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ORIGINAL ARTICLE

Hypericum perforatum chronic treatment affects cognitive parameters and brain neurotrophic factor levels

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Objective: To evaluate the effects of *Hypericum perforatum* (hypericum) on cognitive behavior and neurotrophic factor levels in the brain of male and female rats.
Methods: Male and female Wistar rats were treated with hypericum or water during 28 days by gavage. The animals were then subjected to the open-field test, novel object recognition and step-down inhibitory avoidance test. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell-line derived neurotrophic factor (GDNF) levels were evaluated in the hippocampus and frontal cortex.
Results: Hypericum impaired the acquisition of short- and long-term aversive memory in male rats, evaluated in the inhibitory avoidance test. Female rats had no immediate memory acquisition and decreased short-term memory acquisition in the inhibitory avoidance test. Hypericum also decreased the recognition index of male rats in the object recognition test. Female rats did not recognize the new object in either the short-term or the long-term memory tasks. Hypericum decreased BDNF in the hippocampus of male and female rats. Hypericum also decreased NGF in the hippocampus of female rats.
Conclusions: The long-term administration of hypericum appears to cause significant cognitive impairment in rats, possibly through a reduction in the levels of neurotrophic factors. This effect was more expressive in females than in males.

Keywords: *Hypericum perforatum*; avoidance memory; recognition memory; BDNF; NGF; GDNF

Introduction

Hypericum perforatum (hypericum), commonly known as St. John's Wort, is a traditional medicinal plant possessing antidepressant activity.[1] A systematic review has shown that the effects of hypericum are similar to those of antidepressants in the treatment of major depressive episodes. In addition, hypericum had fewer side effects than conventional antidepressants.[2] In a study by Linde et al.,[2] the most frequently reported side effects or adverse events were gastrointestinal symptoms, increased sensitivity to light, and skin problems. It is important to emphasize that the most important risk associated with hypericum extracts is the potential for interactions with other drugs.[3]

Some studies have demonstrated that the antidepressant mechanisms of hypericum are linked to the inhibition of serotonin reuptake.[4] Hypericum also has an effect on various neurotransmitter systems via the up-regulation of brain levels of serotonin, norepinephrine, and dopamine.[5,6] Furthermore, this medicinal plant causes down-regulation of D2 receptors and up-regulation of 5-HT2A and BDZ receptors,[7] and can increase the levels of intracellular calcium in presynaptic vagal afferent neurons, which, in turn, leads to a release of higher levels of neurotransmitters.[8] Studies have also reported that hypericum can act as protein kinase C (PKC) blocker,[9] competitively binding to the regulatory domain of PKC.[10]

There is a considerable amount of data in the literature demonstrating that hypericum exerts neuroprotective effects in the brain in distinct ways.[11,12] Hypericum reduces formation of nitric oxide (NO), a pro-inflammatory mediator, by decreasing inducible NO synthase expression in mRNA.[12] An animal model of Parkinson's disease induced by rotenone showed that hypericum extract reduced both neuronal damage and dopaminergic cell death, and caused inhibition of the apoptotic cascade by decreasing the levels of Bax in the brain.[11] In addition, some studies have demonstrated the effects of hypericum against cognitive damage in several different models of chronic stress and Alzheimer's disease.[13-18] However, there is little evidence in the literature demonstrating the long-term effects of hypericum in relation to alterations in memory and neurochemistry.

Neurotrophic factors – including the neurotrophin family, glial cell-line derived neurotrophic factor (GDNF), ciliary neurotrophic factor, nerve growth factor (NGF), and

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brain-derived neurotrophic factor (BDNF)[19] – play an important role in memory and in the development and survival of neurons. NGF is essential for the functional integrity of cholinergic neurons within the central nervous system (CNS).[20] In the CNS, NGF is mainly expressed in the cortex, hippocampus, basal ganglia, thalamus, spinal cord, and retina. BDNF belongs to the NGF family and is widely expressed throughout the CNS. It is the most abundant neurotrophin in the adult brain. BDNF is mainly expressed in the hippocampus and exerts its pro-survival effects by binding to its receptor.[21-23] GDNF was originally identified as a potent neurotrophic factor that promotes the survival of midbrain dopaminergic neurons.[24,25] The neurotrophic effects of GDNF have been related to neuronal atrophy, which causes cognitive deficits. Pertusa et al.[26] demonstrated that the increased expression of GDNF improves cognitive deficits in rats.

It is well described in the literature that depressive episodes decrease brain levels of BDNF,[27,28] whereas antidepressants increase the levels of this neurotrophin.[29,30] Molendijk et al.[29] have demonstrated that treatment with antidepressants or hypericum induces up-regulation of serum BDNF in depressed patients when compared to an antidepressant-free depressed group. NGF and GDNF are also related to mood disorders, and antidepressant activity seems to be associated with the up-regulation of these neurotrophic factors.[31] Previous studies from our research group have demonstrated that antidepressant substances improve cognition and enhance neurotrophic expression in animal models of depression.[32] Therefore, studies evaluating the effects of hypericum on neurotrophic factors are important to improve our knowledge about the mechanisms of action of this plant.

There is some evidence to suggest that males and females have different types of cognitive functions, as well as different levels of estrogen and testosterone in the cortex, hippocampus, and amygdala, which are brain regions responsible for modulating cognition and memory. This might explain the differences in cognition seen between the sexes.[33-35] Therefore, the effects of drugs should be tested in both male and female animals. In a systematic review, Leger & Neill[36] demonstrated an advantage of males regarding working memory in a rat model of schizophrenia. In turn, female rats were at an advantage in terms of visual learning and memory and social cognition. Matyi et al.,[37] who evaluated sex differences in Alzheimer's disease risk and neurotrophin gene polymorphisms, suggest several sex differences in the association with Alzheimer's disease and BDNF gene polymorphisms.

It is possible to evaluate recognition memory in rodents, in particular through the use of object recognition tasks, which measure the spontaneous preference for novel objects in an open field.[38,39] Some previous studies using rodents found that interactions between perirhinal cortex, hippocampus, and medial prefrontal cortex are necessary for recognition memory.[40,41] Aversive memory can also be easily measured in rodents in the inhibitory avoidance task. In this task, the animals learn not to step-down from a platform in order to avoid entering a place where they once received a foot shock. This task relies heavily on the dorsal hippocampus,[42,43] where it uses a sequence of molecular events very remindful of those of long-term potentiation.[43-45]

The objective of the present study was to evaluate the pharmacological effects of administering chronic doses of hypericum on the cognitive behavior and the levels of neurotrophic factors in the frontal cortex and hippocampus of male and female rats.

Methods

Animals

Adult male and female Wistar rats (weighing 250-350 g) were obtained from our breeding colony. They were housed five animals to a cage, with food and water *ad libitum*, and maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) at a temperature of 22±1 °C. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimize the number of animals used and their suffering. The experimental procedures were approval of, the local ethics committee for the use of animals (protocol no. 66/2010 UNESC). Experiments were performed during the day, always at the same time, to avoid circadian variations.

Drugs and pharmacological procedures

Dried hypericum extract containing 0.32% of total hypericin (Vitalis Farmácia de Manipulação, Criciúma, Brazil) was used. The dose of hypericum (300 mg/kg once daily) was based on a previous study by Galeotti et al.[46] Hypericum was suspended in water, prepared immediately before administration, and protected from light during the experimental sessions. The solution was homogenized throughout the administration period.

Eighty animals (40 males and 40 females) were randomly assigned to a treatment or a control group: the treatment group (20 males, 20 females) received hypericum (300 mg/kg once daily) during 28 days, for a volume of 1 mL/kg, administered by gavage once daily. Control animals (20 males, 20 females) received water (1 mL/kg). Behavioral tests were initiated 24 h after the last administration of drug/water.

The same animals were used for the open-field test and novel object recognition test [males receiving water or hypericum (n = 10 animals per group) and females receiving water or hypericum (n = 10 animals per group)]. The step-down inhibitory avoidance task was performed with a different set of males receiving water or hypericum (n = 10 animals per group) and females receiving water or hypericum (n = 10 animals per group). Further details about the test procedures are given below.

Behavioral tests

Open-field arena/test

The apparatus consisted of a brown plywood arena (surface area: 45 × 60 cm) surrounded by 50 cm high walls, three

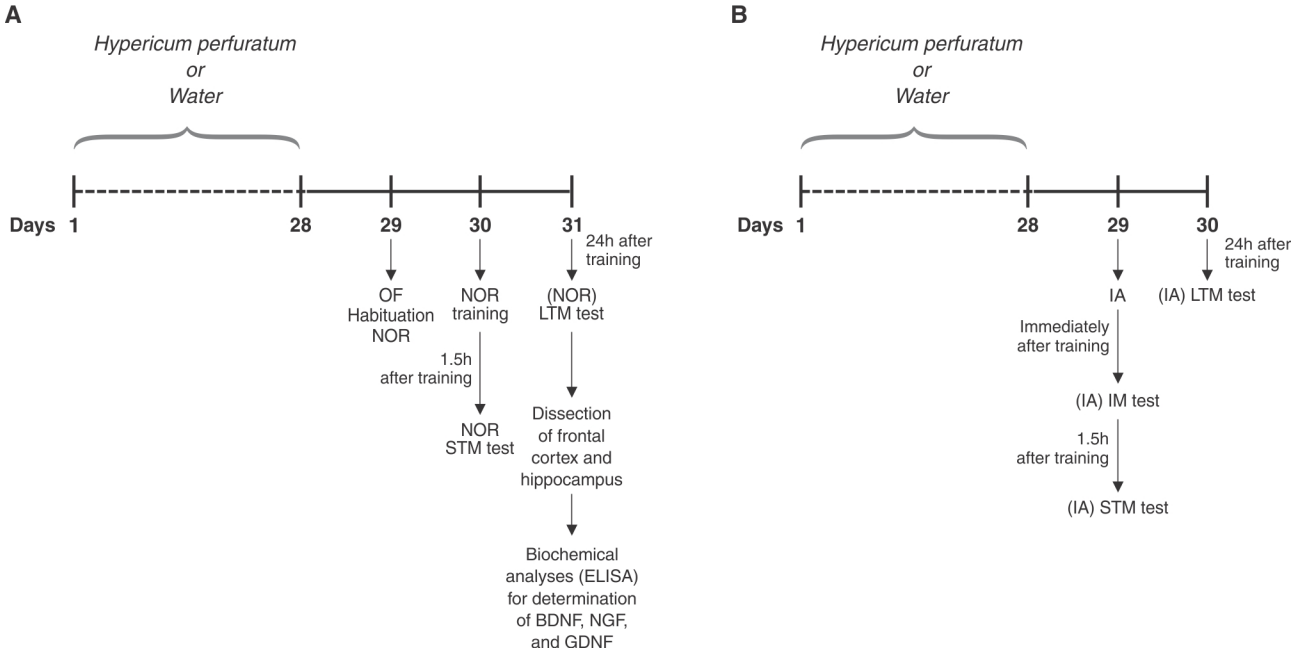

Figure 1 Animals received chronic treatment with *Hypericum perforatum* (hypericum) (20 males, 20 females) or water (20 males, 20 females) once daily during 28 days. 24h after administration of substances, they were submitted to the OF test, NOR test or IA test. A) The same animals were used in the OF and NOR tests [males receiving water or hypericum (n = 10 animals per group) and females receiving water or hypericum (n = 10 animals per group)]. After the NOR test, the animals were killed by decapitation and the brains were removed, with dissection of the hippocampus and frontal cortex for determination of BDNF, NGF, and GDNF levels. Five male and female samples from the water and treatment groups were randomly selected for these analyses. B) A different set of male or female animals receiving water or hypericum (n = 10 animals per group) was used for the IA task. BDNF = brain-derived neurotrophic factor; GDNF = glial cell-line derived neurotrophic factor; IA = inhibitory avoidance; IM = immediate memory; LTM = long-term memory test; NGF = nerve growth factor; NOR = novel object recognition; OF = open-field; STM = short-term memory test.

of which were made of wood. The fourth wall was made of glass. The floor was divided by black lines into nine 15 x 20 rectangles. Individual animals were gently placed on the left rear quadrant and allowed to explore the arena for 5 min. In order to evaluate locomotor and exploratory activities, the number of horizontal (crossings) and vertical (rearings) activities performed by each rat during the 5 min were counted. The open-field test was performed 24 h after the last injection of drug/water (Figure 1). The open-field test was also used for habituation of the animals to the environment for performance of the novel object recognition test.

Novel object recognition (NOR)

The NOR test was performed in the open-field apparatus. Habituation to the apparatus was made during the open-field test, as described above. No objects were placed in the box during the habituation trial. Twenty-four hours after habituation, training was conducted by placing individual rats for 5 min in the open field, in which two identical objects (objects A1 and A2; both being cubes) were positioned in two adjacent corners, at 10 cm from the walls. In a short-term recognition memory test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B, a pyramid with a square-shaped base) object. All objects had similar textures (smooth), colors (blue), and sizes (weight 150-200 g), but distinctive shapes. A recognition index calculated for each animal is reported as the ratio TB/(TA + TB) (TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B). Between trials the objects were washed with 10% ethanol solution. In a long-term recognition memory test given 24 h after training, the same rats were allowed to explore the field for 5 min in the presence of the familiar object A and a novel object C (a sphere with a square-shaped base). Long-term recognition memory was evaluated as described for short-term recognition memory. Exploration was defined as sniffing (exploring the object 3-5 cm away from it) or touching the object with the nose and/or forepaws.

Step-down inhibitory avoidance (IA) task

The step-down IA apparatus consisted of a 50 × 25 × 25-cm plastic box with a front glass wall. The floor was covered by parallel 10-mm bronze bars. The left end of the grid was occupied by a 7-cm wide, 2.5-cm high Formica platform. Individual rats were gently placed on the platform facing the rear wall and their latency to step-down with all four paws on the grid was recorded. In the training session (24 h after the last injection), after stepping down, the animals received a 0.4-mA, 2-s scrambled foot shock and were withdrawn immediately from the cage. There were three test sessions: 1) immediately

after training, to evaluate the immediate memory (IM); 2) 1.5 h after training, to evaluate the short-term memory (STM), and; 3) 24 h after training session, to evaluate the long-term memory (LTM) (Figure 1). In the test sessions, the procedure was repeated, but foot shock was not given. Test session step-down latency was used as a measure of retention. A ceiling of 180 s was imposed on this measure, i.e., animals whose test latency was more than 180 s were considered to have a latency of 180 s, as previously proposed by Gold[47] and Izquierdo & Medina.[43]

Measurement of NGF, BDNF, and GDNF levels

After NOR, the animals undergoing that test were killed by decapitation, and the brains were removed with dissection of the hippocampus and frontal cortex. NGF, BDNF, and GDNF levels were measured in the hippocampus and frontal cortex, using sandwich enzyme-linked immunosorbent assay (ELISA) and commercial kits according to the manufacturer's instructions (NGF and BDNF from Chemicon International Inc., California, USA; GDNF from Biosensis Pty Ltd., California, USA). Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent. Standard curve ranged from 7.8 to 500 pg of BDNF or NGF. Then, plates were wash four times with sample diluents. Anti-BDNF rabbit monoclonal antibody, anti-NGF rabbit monoclonal antibody, or GDNF rat polyclonal antibody diluted 1:1,000 in sample diluent were incubated for 3 h at room temperature (RT). After washing, a second incubation was performed with peroxidase conjugated anti-rabbit antibody diluted 1:1,000 for 1h at RT. After addition of streptavidin-enzyme substrate and stop solution, the amount of BDNF, NGF, or GDNF was determined for absorbance in 450 nm. The standard curve demonstrates a direct relationship between optical density (OD) and BDNF, NGF, and GDNF concentration. Total protein was measured by Lowry's[48] method using bovine serum albumin (BSA) as standard.

Statistical analysis

Data from the open-field and neurotrophin (BDNF, NGF, and GDNF) levels were reported as means ± standard error of mean (SEM) and were analyzed by t test for independent samples. The data obtained in the novel object recognition test were reported as means ± SEM and the differences between groups in this behavioral analysis were verified using repeated measures analysis of variance followed by Tukey's post-hoc tests. The data obtained in the step-down IA task were reported as median ± interquartile ranges (25 and 75). The analysis of IA data was nonparametric because this procedure involved a cutoff score, and the Kruskal-Wallis test was performed followed by Mann-Whitney's U test.

Results

Locomotor and exploratory activities

The open field test was used for assessing locomotor (crossings) and exploratory (rearings) activities in male (Figure 2A) or female (Figure 2B) rats after chronic treatment with hypericum or water. However, no alteration in spontaneous locomotion was observed in either males or females. The number of crossings (male: t-value$_{(17)}$ = -0.436, p = 0.67; female: t-value$_{(21)}$ = 0.807, p = 0.43) and the number of rearings (male: t-value$_{(17)}$ = 0,899, p = 0.38; female: t-value$_{(21)}$ = 0.94, p = 0.35) were also similar.

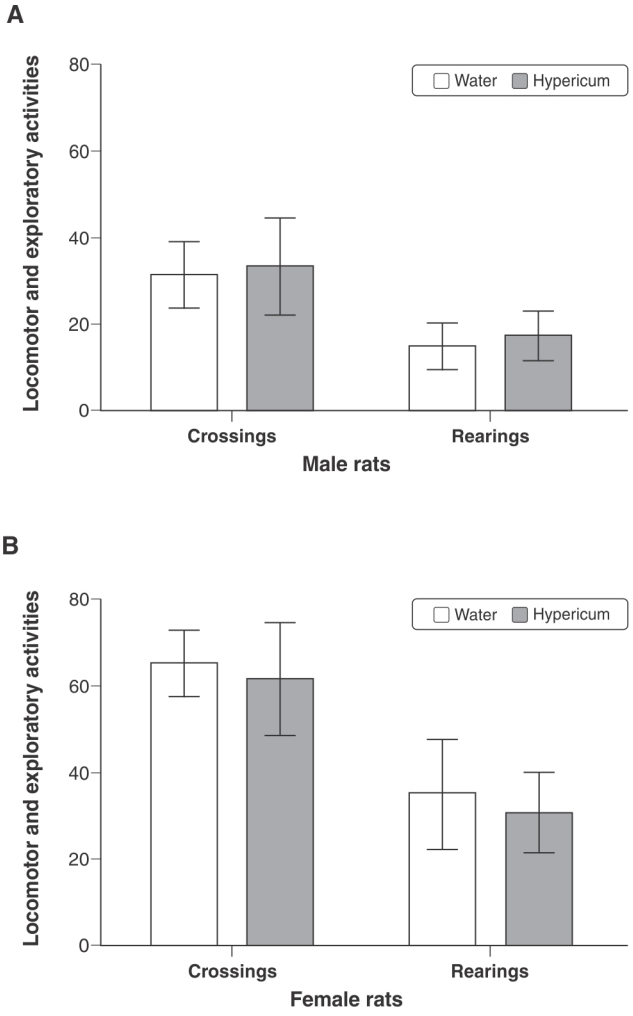

Figure 2 The open field test was used for assessing locomotor (crossings) and exploratory (rearings) activities after chronic treatment with water or *Hypericum perforatum* in (A) males (n=10 animals per group) or (B) females (n=10 animals per group). Data represent the mean ± standard error of mean.

Novel object recognition (NOR)

In all experimental protocols (with both males and females), there were no differences among groups in the total time exploring either object during the retention test trial. Also, there was no significant performance difference among groups in the training trial. These results indicate that pre-training treatment with hypericum did not affect sensorimotor parameters, such as locomotion and motivation. Male (Figure 3A) and female (Figure 3B) animals treated with hypericum showed impaired retention (STM and LTM),

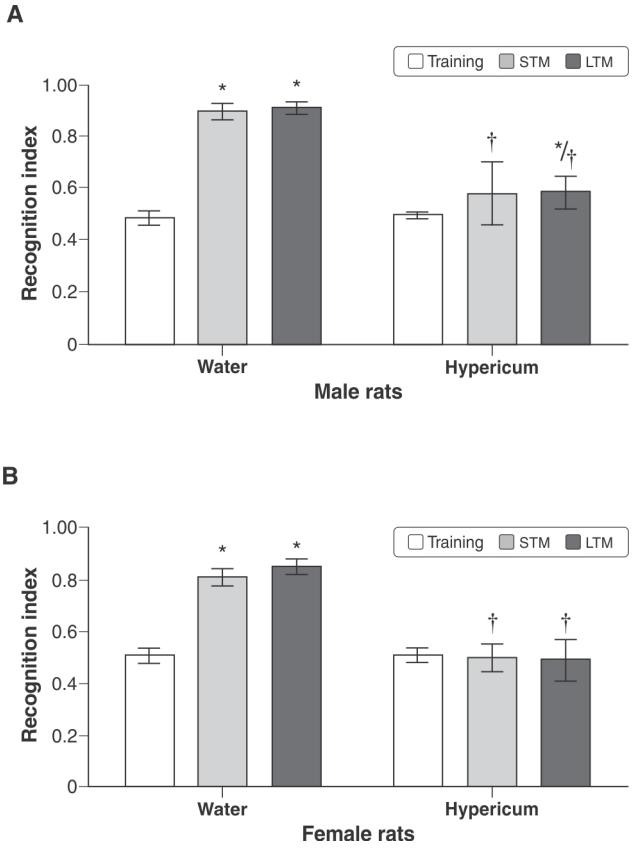

Figure 3 The NOR task was used to evaluate recognition memory. Recognition index for the objects in the training and test sessions after treatment with water or *Hypericum perforatum* appears in (A) for males (n= 10 animals per group) and (B) for females (B) (n= 10 animals per group). Results are presented as means ± standard error of mean of the recognition index. The test session was performed 24 h after the training session. IM = immediate memory; STM = short-term memory; LTM = long-term memory; NOR = novel object recognition. * $p < 0.001$ different from training test. † $p < 0.01$ different from control group, according to one-way repeated measures ANOVA followed by Tukey's post-hoc test.

as shown by decreased preference for the novel object compared to controls given water. Despite a decrease in recognition index during STM and LTM, male rats treated with hypericum showed a significantly higher preference for the novel object during LTM compared to the training trial. However, female rats given hypericum showed no significant preference for the new object during STM and LTM tests.

Repeated measures analysis of variance for drugs administration: males: $F_{(1.14)} = 150.168$, $p < 0.001$; females: $F_{(1.14)} = 251.261$, $p < 0.001$; and for the behavioral repetitions: males: $F_{(2.28)} = 96.21$, $p < 0.001$; females: $F_{(2.28)} = 76.848$, $p < 0.001$.

Step-down inhibitory avoidance (IA) task

In the step-down IA test, control and hypericum animals of both sexes had higher latency in the STM and LTM tests as compared to the training session, indicating acquisition of memory. However, treatment with hypericum induced impairment of the acquisition of memory in males (Figure 4A) and females (Figure 4B), since the latencies in the test sessions were decreased for STM as well as for LTM, as compared with the control group. It should be noted that hypericum administration in female rats also induced impairment of the IM, since there were no statistical differences between training and test latencies.

Data from Kruskal-Wallis test: difference between groups for training (male: chi-square = 0.292, degrees of freedom [df] = 1, p = 0.589; female: chi-square = 2.494, df = 1, p = 0.114), for IM (male: chi-square = 0.494, df = 1, p = 0.482; female: chi-square = 0.991, df = 1, p = 0.320), for STM (male: chi-square = 10.38, df = 1, $p < 0.001$; female: chi-square = 5.258, df = 1, p = 0.022), and for LTM (male: chi-square = 13.223, df = 1, $p < 0.001$; female: chi-square = 0.617, df = 1, p = 0.432).

Data from Wilcoxon test: training session vs. test sessions for control group (training vs. IM of male rats: z = 2.524, p = 0.012; training vs. IM of female rats: z = 2.106, p = 0.035; training vs. STM of male rats: z = 2.521, p = 0.012;

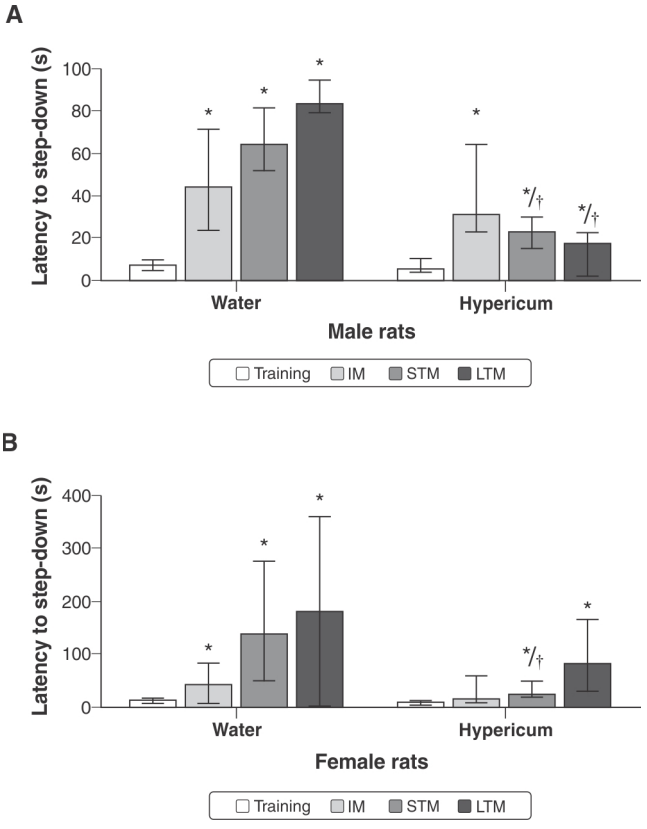

Figure 4 The inhibitory avoidance task was used to evaluate aversive memory. Latency time was recorded in training and test sessions after treatment with water or *Hypericum perforatum* for (A) males (n=10 animals per group) and (B) females (B) (n=10 animals per group). Results are presented as medians ± interquartile ranges (25 and 75). The test session was performed 24 h after the training session. IM = immediate memory; LTM = long-term memory; STM = short-term memory. * $p < 0.001$ different from training test. † $p < 0.01$ different from control group, according to Kruskal-Wallis test followed by Mann-Whitney *U* test.

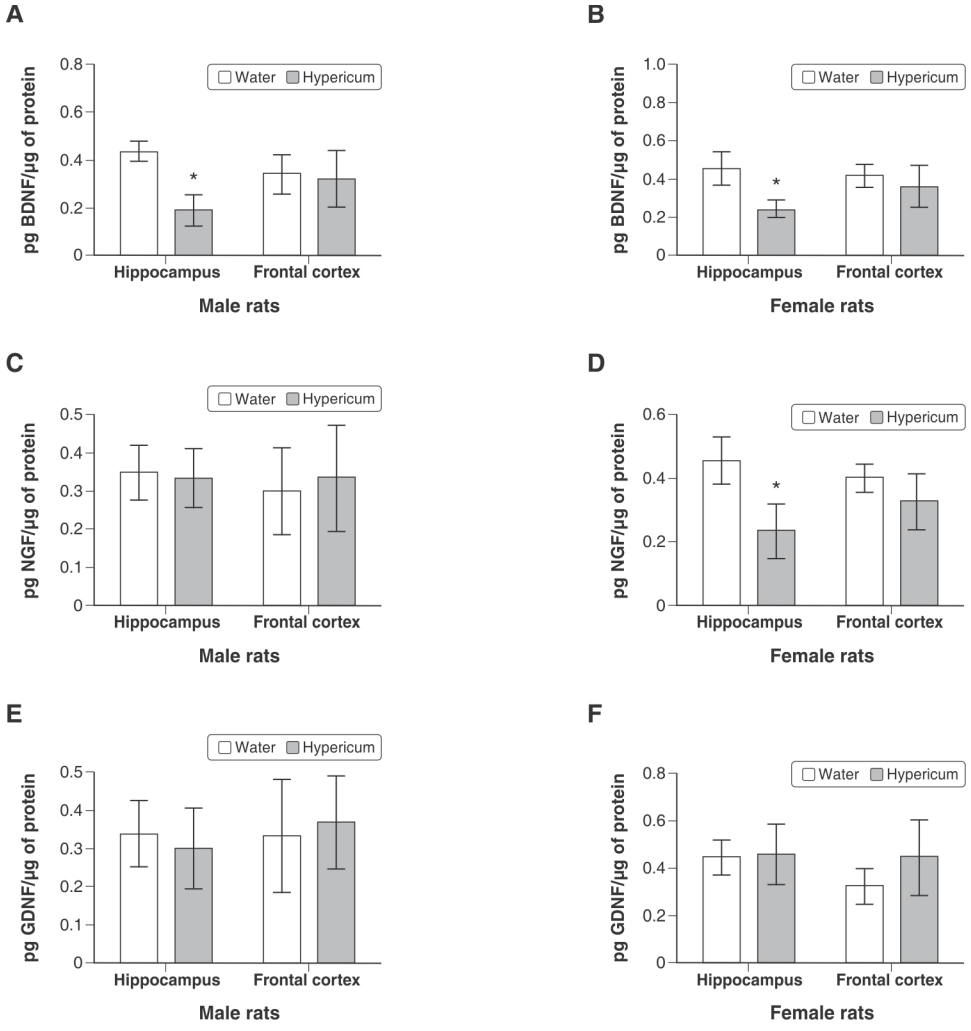

Figure 5 Effects of water or *Hypericum perforatum* on levels of BDNF (A and B), NGF (C and D) and GNDF (E and F) in hippocampus and frontal cortex of male (n = 5 animals per group) (A, C, E) and female (n = 5 animals per group) (B, D, F) rats. Data represent the mean ± standard error of mean. BDNF = brain-derived neurotrophic factor; GNDF = glial cell-line derived neurotrophic factor; NGF = nerve growth factor; * $p < 0.05$ according to Student's *t* test.

training vs. STM of female rats: z = 2.521, p = 0.012; training vs. LTM of male rats: z = 2.524, p = 0.012; training vs. LTM of female rats: z = 2.38, p = 0.017), for hypericum group (training vs. IM of male rats: z = 2.934, p = 0.003; training vs. IM of female rats: z = 1.557, p = 0.119; training vs. STM of male rats: z = 2.936, p = 0.003; training vs. STM of female rats: z = 2.314, p = 0.021; training vs. LTM of male rats: z = 2.803, p = 0.05; training vs. LTM of female rats: z = 2.845, p = 0.004).

Neurotrophic factors levels

The chronic treatment with hypericum decreased BDNF levels in the hippocampus of male (Figure 5A) and female (Figure 5B). Treatment with hypericum decreased NGF levels in hippocampus of female rats (Figure 5C). No changes in NGF levels were observed in the hippocampus or frontal cortex of male rats after chronic administration hypericum (Figure 5D). Hypericum treatment in male (Figure 5E) or female (Figure 5F) rats did not change GDNF levels in the hippocampus or frontal cortex.

Data from *t* test to neurotrophic factors levels: BDNF levels in hippocampus (male: $t\text{-value}_{(8)} = 7.124$, $p < 0.001$; female: $t\text{-value}_{(8)} = 4.820$, $p < 0.01$), BDNF levels in frontal cortex (male: $t\text{-value}_{(8)} = 0.276$, p = 0.79; female: $t\text{-value}_{(8)} = 0.970$, p = 0.36), NGF levels in hippocampus (male: $t\text{-value}_{(8)} = 0.310$, p = 0.76; female: $t\text{-value}_{(8)} = 4.425$, $p < 0.001$), NGF levels in frontal cortex (male: $t\text{-value}_{(8)} = -0.428$, p = 0.68; female: $t\text{-value}_{(8)} = 1.640$, p = 0.14), GDNF levels in hippocampus (male: $t\text{-value}_{(8)} = 0.638$, p = 0.54; female: $t\text{-value}_{(8)} = -0.217$, p = 0.83), GDNF levels in frontal cortex (male: $t\text{-value}_{(8)} = -0.417$, p = 0.69; female $t\text{-value}_{(8)} = -1.544$, p = 0.16).

Discussion

Our results demonstrate that the chronic administration of hypericum did not alter locomotor and exploratory activities as compared to controls. Reis et al.[49] had previously

reported that seven days of treatment with hypericum had no effect on the levels of locomotor activity.[49] In the present study, male and female rats that were chronically treated with hypericum showed impairments in memory retention (STM and LTM) in the object recognition test; however, these memory impairments were more significant in females than in males. Additionally, treatment with hypericum induced impairments in the acquisition of aversive memory (STM and LTM) in both male and female rats in the avoidance step-down test. Interestingly, hypericum administration also induced impairments in the IM in females, but not in male rats.

Our results contradict several studies in the literature relating to improvements in cognitive performance in male rats after administration of hypericum. Trofimiuk et al.[14,18] demonstrated that three weeks of hypericum (350 mg $kg^{[-1]}$) treatment enhanced cognitive performance in male rats performing object recognition, water maze, and Barnes maze tasks following chronic restraint stress or administration of exogenous corticosterone. Widy-Tyszkiewicz et al.[50] also showed that the administration of hypericum (4.3 or 13 μg/kg) in heath rats over a nine-week period decreased the escape latency time and increased the number of platform area crossings in the water maze test, indicating enhanced cognitive performance. Furthermore, Hasanein & Shahidi[51] demonstrated that 30 days of treatment with hypericum (6, 12, or 25 mg/kg) improved the learning parameters in the passive avoidance learning test in an animal model of diabetes mellitus. However, this discrepancy can be explained, at least in part, by methodological differences such as study duration, doses used, and method of administration used for hypericum.

It is important to note that in the present study, hypericum decreased the levels of BDNF in the hippocampus of both male and female rats. BDNF plays an important role in the survival, differentiation, and outgrowth of peripheral and central neurons during development and in adulthood. BDNF has also been shown to play an important role in synaptic plasticity, mainly within the hippocampus.[52,53] Therefore, the memory impairments induced by hypericum might be associated with decreased levels of BDNF in the hippocampus of rats.

It is important to note that the cognitive impairments induced by hypericum were more expressive in females than in males. Some evidence from the literature supports these findings. A preclinical study evaluating cognitive parameters in the offspring of mice treated in the prenatal period with hypericum found that only female offspring had cognitive impairment, which was evaluated in the water maze test. The authors found that adult female offspring exposed to hypericum, rather than to a placebo, required more time to learn the Morris maze task.[54] In the present study, while there BDNF levels seem to have been impaired in both sexes, only females expressed decreased levels of NGF. This neurotrophic factor is responsible for repairing functions in cholinergic neurons within specific regions of the brain that are related to memory formation.[55,56] Thus, these sex-related differences can be explained, at least in part, by the fact that hypericum only decreased the levels of NGF in the hippocampus of female rats.

In the present study, the estrous cycle was not evaluated in female rats during the experiments, even though some studies have demonstrated sex differences in some behavioral response displayed between estrous cycle phases (proestrus, estrous, early diestrus, and late diestrus cycles).[57,58] According to Millad,[59] females in the estrous cycle group (with high estrogen level) presented a quicker extinction of aversive memory than females in the diestrus group (with low estrogen and progesterone levels), suggesting changes of memory and learning tasks in female rats due gonadal hormone influence. Scharfman & MacLusky[60] recently published a review about sex structural brain differences in the hippocampus and the influence of hormonal modulation on cell expression during the estrous cycle. In this same review, the authors suggest that drug treatment could have a variety of effects due to hormonal modulation. However, it is important to emphasize that, in clinical studies, investigators do not assess hormone levels in women's blood in all experiments, and drugs should work in all phases of the cycle. Therefore, the present experiment reflect the procedures of clinical studies. In addition, a clinical study has demonstrated that the menstrual cycle does not always concur with plasma hormone levels.[61]

Regarding limitations, it is important to note that animals can be affected by a novel stimulus in the object recognition task. It could change behavior, provoke stress responses, and elicit approach behavior. Besides, some substances can change the preference for novelty in rats.[38] Therefore, it is plausible to suggest that chronic treatment with hypericum could lead to a change in preference for novelty in rats. However, the present study also detected memory impairment in the inhibitory avoidance task; this supports the notion that chronic hypericum treatment caused cognitive damage. Finally, we did not evaluate the estrous cycle in females during the experiments. However, as demonstrated in a clinical study, the menstrual cycle does not always concur with the hormone plasma levels.[61]

In conclusion, treatment with hypericum induced impairments in both aversive and recognition memories in male and female rats. Chronic administration of hypericum was shown to decrease the levels of BDNF in the hippocampus of both male and female rats when compared to controls. It can be suggested that long-term treatments with PKC inhibitors may lead to significant cognitive impairments by reducing the levels of neurotrophic factors in the brain of rats. Besides, the cognitive damage induced by hypericum was more significant in female than in male rats. This difference can be explained, at least in part, by the fact that treatment with hypericum decreased the levels of NGF only in the hippocampus of females. Additionally, the results suggest that hypericum is more harmful to memory formation in females than in male animals.

Disclosure

The authors report no conflicts of interest.

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