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Original Article

Evaluation of postpartum behaviour in rats treated with *Hypericum perforatum* during gestation

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ABSTRACT

Gestational depression is detrimental to the health of the mother and the offspring and contributes to the appearance of depressive and anxiety symptoms during the postnatal period. Traditional antidepressants have undesirable side effects when utilised during gestation, but *Hypericum perforatum* has been characterised as an efficient and safe antidepressant that prevents the recurrence of symptoms. This study verified the effects of *Hypericum perforatum* on the behaviour of Wistar rats that were treated during gestation and evaluated 10 and 60 days post-treatment. Pregnant Wistar rats were divided into four groups of ten animals each: one control group that received distilled water and three treatment groups that were treated orally with 36, 72 or 144 mg/kg *Hypericum perforatum* extract. At 10 and 60 days after parturition and post-treatment, the rats were submitted to the hole-board, the tail suspension, and the forced swim tests. The animals treated with 144 mg/kg *Hypericum perforatum* exhibited greater head-dipping activity in the hole-board test and reduced immobility in the tail suspension and forced swim tests, suggesting less anxiety and depression 10 and 60 days post-treatment. The results indicated that treating rats with *Hypericum perforatum* during the gestational period decreased depressive behaviour and anxiety 10 and 60 days post-treatment.

Introduction

Depression is a worldwide public health problem and is considered one of the leading causes of disability. In 2001, approximately 154 million people were affected by depression, and it is estimated that it will become the most common illness in the world by 2030 (WHO, 2001). In women, depression is even more disturbing when it occurs during gestation; according to certain authors, the neuroendocrine changes that occur during gestation favour the development of depressive disorders (Campagne, 2004; Steiner et al., 2003).

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Gestational depression modifies the maternal environment and severely damages the health of the mother and the offspring (Field et al., 2008; Oberlander et al., 2006; Rosen et al., 2007). The occurrence of a depressive disorder during gestation can contribute to mood and anxiety disorders during the postpartum period because symptoms expressed during gestation can become exacerbated postpartum (Bloch et al., 2003; Brockington, 2004; Riecher-rössler and Steiner, 2005; Steiner et al., 2003). Because of this, it is imperative to identify an efficient treatment for depression that can be safely administered to pregnant women and that decreases both the recurrence of symptoms and the risk of mood and anxiety disorders in the puerperium.

Traditional antidepressants exert diverse side effects and can be toxic to the child when taken during gestation. Furthermore, they are found in the placenta, amniotic fluid and breast milk, suggesting that they should be utilised cautiously by pregnant women (Dodd et al., 2006; Hostetter et al., 2000). Sufficient data in the literature have provided evidence that there is no relationship between the use of these medications for the treatment of gestational depression and a reduction in the recurrence of depressive and anxiety symptoms in the puerperal period (Dodd et al., 2006; Newport et al., 2001).

Hypericum perforatum L., Hypericaceae, commonly known as hyperic or St. John's wort, is a proven antidepressant that has no indications of severe side effects. In mice treated during gestation, there is no toxicity to the offspring and no interference with motor and behavioural development, as assessed immediately after weaning (Bahls, 2001; Brattstrom, 2009; Cada et al., 2001; Nepomuceno et al., 2005). In addition, *H. perforatum* (HP) has a long-lasting effect and prevents the recurrence of symptoms even after the treatment has ended, indicating that HP should be regarded as an efficient and safe alternative to treat depression during gestation (Singer et al., 2011). Phytochemical studies have characterised the presence of various substances in HP, including flavonoids, naphthodianthrones (such as hypericin) and hyperforin, a prenylated phloroglucinol derivative that is believed to be the primary active constituent responsible for the antidepressant and anxiolytic properties of the herbal extracts (Singer et al., 1999).

Based on the antidepressant activity of HP, its possible long-lasting effects and its ability to prevent the recurrence of symptoms, this study was designed to evaluate the postpartum behaviour of Wistar rats 10 and 60 days after the end of HP treatment during gestation.

Materials and methods

Plant material

A standard dry extract of *Hypericum perforatum* L., Hypericaceae, containing 0.3% hypericin was imported from China, identified by the deposit number 20100913, and prepared by Mbpharma Manipulações LTDA as lot 10124778E.

Assays

Pregnant Wistar rats (*Rattus norvegicus* Berkenhout, 1769) were obtained from the vivarium of the Centro de Biologia da Reprodução, Federal University of Juiz de Fora, at 90 days of age and were housed in polypropylene cages (40 x 30 x 16 cm) containing five animals each. The animals were housed under standard laboratory conditions, with a controlled temperature of 23 ± 2°C and a 12 h light/dark photoperiod, with the light period beginning at 6 AM. The dams had free access to water and were fed rat chow pellets (Purina). They were deprived of feed 60 min before the beginning of the tests. After parturition, each female and her offspring were placed in individual cages and then separated after weaning.

The dams were distributed into four groups of ten pregnant rats each: three treatment groups and one control group. During gestation, the rats in the treatment groups received oral (gavage) doses of the aqueous extract of HP at 36 mg/kg (T1), 72 mg/kg (T2), and 144 mg/kg (T3) once daily. The volume ranged from 0.4 to 0.6 ml, depending on the weight of the pregnant rat. The rats in the control group (C) received distilled water during the same time period. The doses were chosen based on information available in the literature. HP action in the rat central nervous system (CNS) was previously observed at doses higher than 30 mg/kg (Crupi et al., 2011).

The dams were examined 10 and 60 days after parturition. Ten days after the end of the treatment, the animals were submitted to the hole-board test to evaluate anxiety and to the tail suspension test to evaluate depression. Sixty days after parturition, the rats were once again submitted to the same tests, along with the forced swim test to confirm depressive behaviour. The forced swim test was not performed 10 days after parturition to avoid any interference of hypothermia with breast-feeding. The rats were weighed daily during the treatment period.

Hole-board test

The hole-board test is frequently utilised to screen for effects of psychotropic drugs on the exploration and spatial learning behaviour of a rodent. When placed in a new environment, the natural tendency of an animal is to explore the holes by plunging its head in and out of the hole a few times. This test enables an assessment of the effects of psychotropic drugs on head dipping and exploration activity and is based on the assumption that head-dipping is inversely proportional to anxiety (Boisser et al., 1976; Carlini and Mendes, 2001; Mattei and Carlini, 1995; Saitoh et al., 2006). In addition, locomotor activity (movements with dislocation) and stereotypical and exploratory movements in the holes are also parameters that reflect the anxiety level (Casarrubea et al., 2009).

The hole-board apparatus (model LE 8811, from Panlab s.l) consisted of a transparent acrylic box with sixteen equidistant holes on the surface and photoelectric cells on the side plates that were capable of conducting an automated reading animal movement, thereby preventing possible human error during observation. Each animal was placed in the centre of the plate once and was observed for five minutes. The number of head-dips into the holes, the locomotor activity (displacement), and the stereotypical movements were registered.

Tail suspension test

The tail suspension test was described by Steru et al. (1985) and is widely utilised in preclinical studies to evaluate the antidepressant potential of novel compounds. This test is based on the behavioural response of rodents to a stressful situation that is perceived as hopeless; animals subjected to the short-term, inescapable stress of being suspended by their tail develop an immobile posture. Antidepressant medications reverse the immobility and promote escape-related behaviour. The animals were individually suspended by the tail at a height of 50 cm for five min, and depression was measured based on immobility. Increased total immobility time and a shorter duration of attempting to escape represent more intense depressive behaviour (Teixeira-Silva et al., 2006).

Forced swim test

The forced swim test described by Porsolt et al. (1977) is one of the most utilised methods for screening antidepressants. This test is based on the assumption that animals try to escape from an aversive stimulus, providing information about their emotional status. The rats underwent a pre-test protocol: they were individually forced to swim in polypropylene cages (60 × 40 × 40 cm) containing water at a temperature of 25 ± 1° C for 15 min as the dangerous situation. The data from this phase were not included in the statistical analysis. On the next day, the animals were placed in a transparent glass cylinder filled with water for 5 min and forced to swim. Their behaviour was recorded by registering the time taken by the animal to stop struggling and swimming (latency time) as well as the duration of immobility. An increased immobility time during this test is an accurate indication of the effects of antidepressants (Carlini and Mendes, 2001). After each trial, the water was changed.

This project was approved by the Ethical Committee in Animal Experimentation of the Universidade Federal de Juiz de Fora (protocol number 003/2011).

Statistical analysis

One-way Analysis of Variance (ANOVA) was utilised for statistical comparisons of the differences between the test groups (α = 0.05), and the results were expressed as the mean ± standard error for n = 10 observations. For the *in vivo* studies, n represented the number of animals studied. Significant interactions were analysed using post-hoc ANOVA with an adjusted *p value* (Sokal and Rohlf, 1995).

Results

No significant changes in the weight of the control and HP-treated animals were observed during gestation. In the hole-board test, the average head-dipping activity was considerably higher 10 and 60 days after parturition in animals treated with 144 mg/kg HP extract and 60 days after parturition in animals treated with 72 mg/kg HP extract compared with the average head-dipping activity of control animals (Fig. 1). Ten days after parturition, the average amount of locomotor activity (movements with dislocation) was considerably higher and the average number of stereotypical movements was significantly lower in animals treated with 144 mg/kg HP compared with control animals (Fig. 2). The average number of stereotypical movements was also lower sixty days after parturition in animals treated with 144 mg/kg HP (Fig. 3).

In the tail suspension test, the animals treated with 144 mg/kg HP extract exhibited a significant increase in the latency time and a significant reduction in the total immobility time at both ten and sixty days post-treatment. The animals that received 72 mg/kg HP had a statistically lower total immobility time than the control animals at the sixty-day evaluation (Figs. 4 and 5).

In the forced swim test, the average latency time for immobility was considerably higher in animals treated with 72 or 144 mg/kg HP extract, whereas the total immobility time was considerably lower in these same animals (Fig. 6).

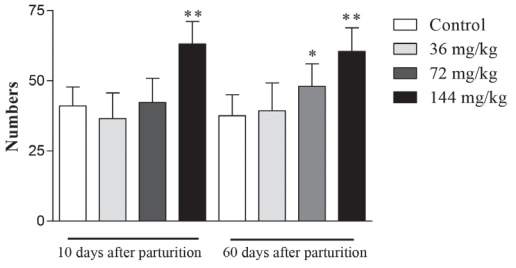

Fig. 1 - The number of head-dips by control and HP-treated rats in the hole-board test. The data are presented as the average ± standard deviation (n = 10; * $p < 0.05$; ** $p < 0.001$).

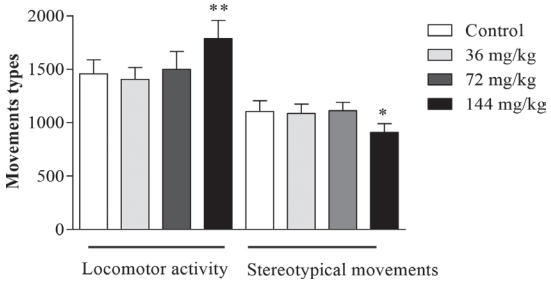

Fig. 2 - The amount of locomotor activity and number of stereotypical movements by the control and HP-treated rats during the spontaneous activity test 10 days post-treatment. The data are presented as the average ± standard deviation (n = 10; * $p < 0.05$; ** $p < 0.01$).

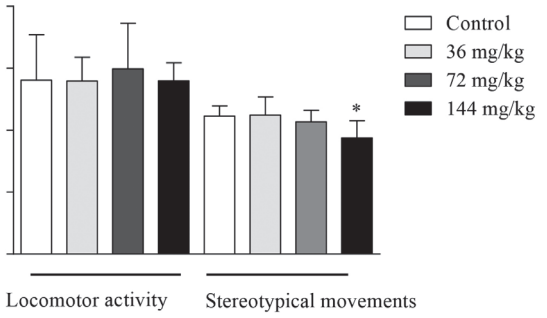

Fig. 3 - The amount of locomotor activity and the number of stereotypical movements by the animals during the spontaneous activity test 60 days post-treatment. The data are presented as the average ± standard deviation (n = 10; * $p < 0.05$).

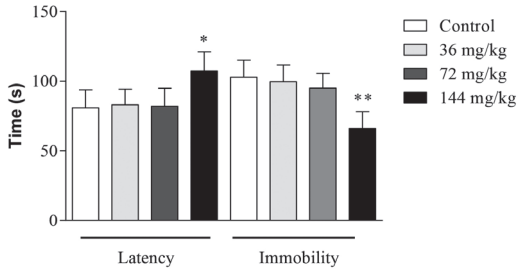

Fig. 4 - The latency time (s) to initiate immobility and the total immobility time during the tail suspension test in control and HP-treated rats evaluated 10 days after parturition. The data are presented as the average ± standard deviation (n = 10; * $p < 0,01$; ** $p < 0,001$).

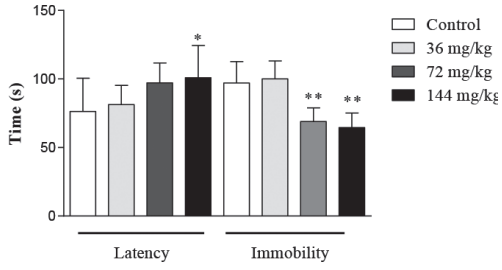

Fig. 5 - The latency time (s) to initiate immobility and the total immobility time during the tail suspension test in control and HP-treated rats evaluated 60 days after parturition. The data are presented as the average ± standard deviation (n = 10; * $p < 0.05$; ** $p < 0.001$).

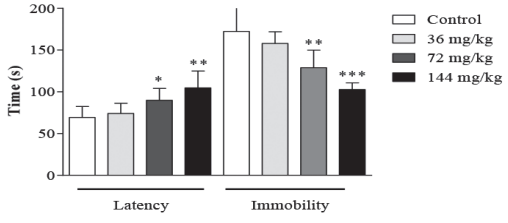

Fig. 6 - The latency time (s) to initiate immobility and the total immobility time during the forced swim test in control and HP-treated rats evaluated 60 days after parturition. The data are presented as the average ± standard deviation (n = 10; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Discussion

Any treatment that might cause toxicity must be utilised with restrictions or should not be prescribed during gestation. Body weight reductions in treated animals are indicative of organismal toxicity (Hiremath et al., 1997); however, alterations in food intake, and consequently in body weight, may also indicate a depressive episode (Beck and Brad, 2008; Chahoud et al., 1999). In this study, no significant differences in body weight were observed in rats treated with HP during gestation, suggesting that this compound was not toxic (Nepomuceno et al., 2005).

Gestational depressive disorders can lead to the manifestation of mood and anxiety disorders during the postpartum period, with an intensification of anxiety symptoms (Riecher-rössler and Steiner, 2005; Schmidt et al., 1997). Nonetheless, these symptoms, when secondary to depression, tend to disappear after treating the disorder (Ribeiro, 2007). Therefore, the evaluation of depressive and anxiety behaviours may be helpful for the diagnosis, treatment, and monitoring of depression.

In the hole-board test, increased animal motion and head-dipping activity indicate greater exploratory behaviour, whereas decreased stereotypical movements reflect the fear of the animal in the unfamiliar environment. All these behaviours reflect less anxiety. Therefore, the parameters examined in this study suggested that the highest dose of HP extract (144 mg/kg), when administered during gestation and evaluated ten and sixty days post-treatment, exerted an anxiolytic effect in the animals as evidenced by their increased locomotor and head-dipping activity and reduced stereotypical movements (Carlini and Mendes, 2001).

In preclinical studies evaluating the potential antidepressant properties of natural or synthetic substances, the forced swim and the tail suspension tests have been utilised to assess the emotional status of animals via behavioural despair (Almeida, 2006). In this study, the increased latency time and reduced immobility suggested less depression in animals treated with the two higher doses of HP extract (72 and 144 mg/kg) and evaluated ten and sixty days post-treatment.

The reduced depression and anxiety in the post-partum period in rats treated during gestation confirmed the chronicity of the effects of HP. Patients treated with HP presented with fewer depressive symptoms and experienced a longer time to recurrence of such symptoms compared with patients treated with citalopram, a selective serotonin reuptake inhibitor that is traditionally used to treat depression. On average, the symptoms reappeared 1817 days after terminating the treatment (Singer et al., 2011).

HP primarily acts by inhibiting the reabsorption of the neurotransmitters serotonin, noradrenaline, and dopamine. Therefore, the increased concentration of these substances in the synaptic cleft justifies the antidepressant effects. When HP is used for a long time, adaptations occur in intracellular messengers and receptors involved in neurotransmitter activity (Franklin, 2003; Ruedeberg et al., 2010) and in hippocampal neurons (Crupi et al., 2011). These data explain the effects observed in this study even after the treatment had ended.

The results obtained in this study demonstrated that 72 and 144 mg/kg HP extract interfered with animal behaviour, even ten and sixty days post-treatment, thereby providing further support for its chronic effects. The results suggested that treating rats during gestation with HP inhibited the development of post-partum depressive and anxiety symptoms, which are often initiated by depression during gestation. Nonetheless, more studies are necessary to confirm the effects and the mechanisms of action of HP.

Authorship

VAV, LRS and LVC performed the laboratory work and the data analyses and wrote the paper. RCSS and VMP designed the study, supervised the laboratory work and contributed to the critical reading of the manuscript. All the authors have read the final manuscript and approved the submission. MOG contributed by critically reading the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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