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Original Article

Rats treated with *Hypericum perforatum* during pregnancy generate offspring with behavioral changes in adulthood

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ABSTRACT

Drugs used in the treatment of depression can cross the placenta giving rise to questions regarding the effects these drugs exert on the fetus. *Hypericum perforatum* L., Hypericaceae, is a natural product used to treat depression. However, information about its toxicity and the occurrence of alterations in the central nervous system development of the offspring is scarce. This work assessed the behavior of adult male rats born from mothers treated with *Hypericum* extract during gestation and analyzed the fluorescence of the extract in different organs of mothers and fetuses. Male pups were divided into three treated groups, corresponding to the administration of the *Hypericum* extract to mothers at the dose levels of 36 mg/kg, 72 mg/kg and 144 mg/kg, and one control group in which the mothers received distilled water. At 90 days of age, the offspring underwent the following tests: rotarod, pentobarbital-induced sleep time, elevated plus maze, hole-board and forced swimming test. The observed fluorescence indicated the presence of the extract in all tissues analyzed. The obtained results suggest lasting changes in the performances displayed in the CNS, depression and anxiety tests, indicating that the use of *Hypericum* during gestation could interfere with the behavioral development of the offspring reducing anxiety and depression when they become adults. We suggest that these alterations are associated with the reprogramming of the brain regions related to changes in emotional reactivity.

Introduction

Depression is the most prevalent mental disorder in pregnant women, whose incidence is progressively increasing over the years (Bennett et al., 2004; Pereira and Lovisi, 2008). The neuroendocrine changes that take place during gestation are considered risk factors for the appearance of depressive disorders, and contrary to popular belief, the gestational period does not protect the women's mental health (Campagne, 2004; Camacho et al., 2006). The gestational depressive disorder alters the maternal environment and produces severe damages to the health of the mother and child, such as decreased quality of life, puerperal depression and anxiety setting, obstetric complications, gestational hyperemesis, premature birth, low fetus weight, increased hospitalization in the neonatal intensive care unit and interference in the newborn's development (Oberlander et al., 2006; Field et al., 2008). All these factors emphasize the importance of the treatment of gestational depression as a prenatal care measure.

The tricyclic antidepressants, the monoamine oxidase inhibitors (MAOI) and the selective serotonin reuptake inhibitors (SSRI) are different classes of synthetic medications used in the treatment of depression during pregnancy known to improve 60 to 70% of the symptoms in 30 days. However, these drugs also produce undesirable side effects, including sexual dysfunction, hallucinations, delirium, risk of maniac episodes, gastrointestinal disorders, anticholinergic effects, sedation and intoxication (Souza, 1999). In addition, preclinical studies have shown that they increase the risks of teratogenicity, can cross the placenta and can be found in the amniotic fluid (Hostetter et al., 2000).

The tolerance developed by patients after chronic exposure to some antidepressants and anxiolytics is mainly characterized by the reduction in the number of serotonin (5-HT), noradrenalin (NOR) and gamma-aminobutyric acid (GABA) receptors. Therefore,

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their use by pregnant women could affect fetal neurodevelopment, since the formation of the central nervous system (CNS) continues for an extended period postnatally (Murrin et al., 2007).

Hypericum perforatum L., Hypericaceae, is popularly known as Hyperic or St John's wort and is a well-known antidepressant (Schmidt and Butterweck, 2015) that also shows antinociceptive and anticonvulsant effects (Galeotti et al., 2014) and others (Linde, 2009). *Hypericum perforatum* (Hp) can be found distributed across several continents: Europe (Germany, Russia, Poland), West Asia, North Africa, North America, South America and Australia. It is considered efficacious and safe when compared to synthetic medications (Linde et al., 2008, 2009; Howland, 2010; Nahas and Sheikh, 2011), since its use does not cause the typical side effects of psychoactive substances such as sedation, memory loss, addiction and (Brattstrom, 2009) intolerance. No teratogenic effects have been reported when taken by pregnant women, however its use should be done with caution as the action of *H. perforatum* on the maternal and fetal organism as well as the future consequences on the behavior of the offspring have not been fully elucidated (Tschudin and Lapaire, 2005; Dugoua et al., 2006).

Hypericum perforatum extract is a phytocomplex composed of various substances, including hypericin, pseudohypericin, hyperforin, flavonoids (quercetin, hyperoside and rutin), tannins and procyanidins. Various concentrations have been identified in the commercial products of the extract (Bergonzi et al., 2001), however what defines the standard extract is the concentration of hypericin which should be 0.3% (Muller et al., 1997). The antidepressive effect of Hp extract can be attributed to the isolated or synergistic action of the substances present in it (Reichling et al., 2003), but several reports indicate that hypericin, pseudohypericin, the flavonoids and hyperforin may be the main components responsible for this effect (Xu et al., 2005; Borrelli and Izzo, 2009). In particular, hyperforin is known to inhibit the reuptake of 5-HT, NOR, dopamine and glutamate and to interfere with the neuronal efflux and influx of electrolytes (Bouron and Lorrain, 2014).

Owing to the knowledge that Hp extract presents fluorescent constituents, such as hypericin and pseudohypericin (Draves and Walker, 2000), this work employed the *in vivo* imaging system to determine the biodistribution of the extract on the placenta and on the maternal and fetal organisms. In addition, this work also evaluated the behavior of adult male rats born from mothers treated with Hp extract during gestation.

Material and methods

Plant material and extract

The standard dry extract of *Hypericum perforatum* L., Hypericaceae, containing 0.3% of hypericin, was imported from China and identified by number of deposit 20100913. The extract was then prepared by Mbpharma Manipulações Ltda (Matias Barbosa, Brazil), lot 10124778E and stored at temperatures between 15 °C e 25 °C in a dark glass vial.

Animals and treatment

One-day pregnant Wistar rats (*Rattus norvegicus*) (90 days old), were obtained from the vivarium of the Center for Reproductive Biology of the Federal University of Juiz de Fora and housed in polypropylene cages (40 × 30 × 16 cm), containing five animals each. They were kept under standard laboratory conditions, with 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 on rat chow pellets (Nuvilab® -Paraná, Brazil).

The dams were randomly distributed into four groups of ten pregnant rats: three treated groups and a control one, and underwent treatment during the gestational period once daily. The treated groups received orally (gavage) the aqueous extract of Hp at doses of 36 mg/kg (T1), 72 mg/kg (T2), and 144 mg/kg (T3) of body weight. The control group (C) received distilled water during the same period. Treatment of mothers was discontinued after delivery. The choice of doses was based on the information available in the literature, taking into account that the action of Hp in the central nervous system (CNS) of rats was previously observed at doses higher than 30 mg/kg (Crupi et al., 2011).

One day before giving birth, the rats were placed in individual cages. After weaning, the male offspring were divided into four groups: T1, T2, T3 and C, corresponding to the treatments received by their respective mothers. At 90 days of age all male pups were weighed and ten animals from each group, comprising random selection of one descendent from each mother, were subjected to the rotarod test and to the pentobarbital-induced sleep time test seven days later. Ten other males from each group were subjected to the elevated plus maze test and a week later to the forced swim test. In a similar way, ten other animals were evaluated in the hole-board test. The female pups were studied separately and the results will be presented elsewhere.

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

Motor performance test (rotarod test)

Motor performance was measured as time spent walking on a rotating rod (7 rpm) in just one trial, *i.e.*, after falling from the bar the animal was returned to the cage. To this effect, the animals were submitted to a preselection 24 h before testing and only those remaining on the revolving bar of the rotarod for at least 60 s in one out of three trials were selected (Kannan et al., 2013). The equipment used was a rotarod Panlab s.l. mod. LE 8200 (Barcelona, Spain).

Pentobarbital-induced sleep time

The animals were injected *i.p.* with sodium pentobarbital (Syntec, Hypnol^{R}; 40 mg/kg, Juiz de Fora, Brazil) and the latency (in seconds) – interval between the pentobarbital administration and the beginning of the sleeping time – and the time (in seconds) between loss and recovery of the righting reflex were recorded (Wambebe, 1985)

Elevated plus maze test

The elevated plus maze apparatus (Novalab; Ribeirão Preto, Brazil) consisted of four arms (50 cm) elevated 39 cm above the floor. Each arm was positioned at 90° relative to the adjacent arms and all arms were connected through a central area (5 × 5 cm), forming a plus sign. Each rat was placed at the center of the maze facing one of the open arms. The time spent (in seconds) in the open arms was recorded for 3 min. Entry into an arm was defined as the animal having crossed with all four paws the dividing line between the central area and the arm (Herrera-Ruiz et al., 2008; Grundmann et al., 2009; Han et al., 2009). After each trial, the plus maze was carefully cleaned with 10% ethanol solution.

Hole-board test

The hole-board apparatus (Panlab s.l. mod. LE 8811, Barcelona, Spain) consisted of a transparent acrylic box with sixteen equidistant holes on the surface with photoelectric cells on the side plates.

These cells were capable of conducting an automated reading of the animals' movements, preventing possible human errors during observation. Each animal was placed once at the center of the plate facing away from the observer and the number of head-dipping activity in the holes, locomotor activity (ambulation), and stereotypical movements were registered for 5 min (Han et al., 2009). After each trial, the hole-board was carefully cleaned with 10% ethanol solution.

Forced swim test

The rats were placed in a transparent glass cylinder (height: 50 cm; diameter: 20 cm) filled with water and forced to swim for 5 min. The water was changed after each trial. The following parameters were evaluated for behavior analysis: time taken by the animal to stop struggling and swimming (latency time) and duration of immobility. This test is based on the assumption that animals try to escape from an aversive stimulus, providing information on their emotional status (Porsolt et al., 1977). Therefore, an increase in the immobility time gives relevant information about the effects of antidepressants (Carlini and Mendes, 2011).

Biodistribution assay

The *in vivo* imaging system (IVIS) was employed to visualize the biodistribution (quantification of fluorescence intensity) of Hp extract in the pregnant females and their respective fetuses. Saline solution (C) and Hp extract at the dose levels of 36 mg/kg (T1), 72 mg/kg (T2), and 144 mg/kg (T3) were administered daily, by gavage, to pregnant females from day 18 to 21 of gestation. Each experimental group consisted of four females ($n = 4$) and after birth, three male pups were randomly selected ($n = 12$ offspring per group). Three hours after the last treatment, the females were killed by deep anesthesia with ketamine (90 mg/kg)-xylazine (10 mg/kg) solution followed by blood collection by cardiac puncture (Wolfensohn and Lloyd, 1994). The pups were removed by a cesarean section and then the mothers underwent laparotomy for removal of retroperitoneal fat, kidney, heart, liver, spleen, lung, brain and the placenta. In the fetuses, liver and brain were dissected out. The isolated tissues and organs were then washed in saline solution and were immediately imaged in IVIS Kodak Image Station 4000MM PRO (Carestream Health Inc., Rochester, USA) equipped with a CCD (charge-coupled device) camera. For the fluorescence imaging, the machine was configured for 610 nm excitation, 700 nm emission filters, 3 min exposure and binning of 2×2. The acquired images (mean intensity of each sample) were analyzed with the Carestream MI Application version 5.0.2.30 software and later compared to control animals, which presented normal values of fluorescence = 1 (a.u. = 1).

Statistical analysis

One-way Analysis of Variance (ANOVA) followed by the Dunnett's test was used whenever the data showed homoscedastic distribution. Non-homoscedastic data were analyzed using the Dunnett's T3 test. To compare the results of all replicates, the data were normalized by dividing the values of each group by its respective control group value. The results were expressed by mean ± standard deviation of mean (S.D.M.) of "$n = 10$" observations. The difference between groups were considered significant when $p < 0.05$. The tests were performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego USA (Sokal and Rolf, 1994).

Table 1
Mean weight (g) of control and hyperic-treated offspring (F1).

Groups	Body weight (g)
Control	349.4 ± 10.0
T1 (36 mg/kg)	332.7 ± 29.0
T2 (72 mg/kg)	340.5 ± 30.1
T3 (144 mg/kg)	340.0 ± 25.6

Values are mean ± S.D.M. (each group $n = 30$) (ANOVA test, followed by the Dunnett's test).

Results

General parameters assessment of antidepressant activity of Hypericum perforatum

The weight of male offspring (F1) at 90 days of age did not change significantly between control and treated groups (Table 1).

In the rotarod test, the time spent walking on the rotating rod was significantly reduced in the offspring from groups in which the mothers were treated with 72 mg/kg (T2) and 144 mg/kg (T3), while in the pentobarbital-induced sleep time, latency was not altered but the animals born from mothers treated with the highest dose (T3) exhibited prolonged sleep time when compared to control animals (Table 2).

Assessment of anxiolytic activity of Hypericum perforatum

In the elevated plus maze test, the results indicate that the animals born from mothers treated with 144 mg/kg (T3) of the extract showed a significant increase in the amount of time spent in the open arms when compared to control values. Similarly, in the hole-board test, the number of head dips and ambulation were significantly increased in the T3 group (Table 3).

Evaluation of antidepressant activity of Hypericum perforatum

In the forced swim test, increased latency time and reduced immobility time were observed in the offspring from group T3 (Table 4).

Table 2
Effect of the rotarod and the pentobarbital-induced sleep time in control and hyperic-treated offspring (F1).

Groups	Time on rod (s)	Sleep time (min)
Control	72.7 ± 9.4	51.3 ± 5.7
T1 (36 mg/kg)	73.6 ± 14.7	51.7 ± 7.4
T2 (72 mg/kg)	56.7 ± 22.9[a]	53.4 ± 6.2
T3 (144 mg/kg)	35.3 ± 8.9[a]	63.9 ± 9.5[a]

Values are mean ± S.D.M. ($n = 10$).
[a] $p < 0.05$ (ANOVA test, followed by the Dunnett's test).

Table 3
Effect of the elevated plus maze and the hole-board in control and hyperic-treated offspring (F1).

Groups	Mean time spent in the open arms (s)	Head dip[a]	Ambulation[a]
Control	73.2 ± 22.3	28.9 ± 8.4	1117.9 ± 258.4
T1 (36 mg/kg)	71.6 ± 24.3	31.0 ± 10.1	1093.0 ± 197.6
T2 (72 mg/kg)	81.2 ± 24.1	53.3 ± 11.6	1213.2 ± 404.3
T3 (144 mg/kg)	87.7 ± 23.3[b]	53.1 ± 9.8[b]	1550.5 ± 400.9[b]

Values are mean ± S.D.M. ($n = 10$).
[a] Values represent the mean of movements quantity (ANOVA test, followed by the Dunnett's test).
[b] $p < 0.05$.

Table 4
Effect of the forced swim in control and hyperic-treated offspring (F1).

Groups	Latency (s)	Immobility (s)
Control	80.2 ± 10.5	109.6 ± 12.2
T1 (36 mg/kg)	79.5 ± 14.9	103.2 ± 15.0
T2 (72 mg/kg)	82.5 ± 18.4	105.9 ± 13.3
T3 (144 mg/kg)	106.4 ± 12.1[a]	61.3 ± 11.2[a]

Values are mean ± S.D.M. ($n = 10$).
[a] $p < 0.05$ (ANOVA test, followed by the Dunnett's test).

Passage of Hypericum perforatum *extract through the placental barrier*

The *in vivo* imaging system showed that the fluorescence intensity was significantly increased in the retroperitoneal fat, heart, lung and liver in the pregnant females treated with 144 mg/kg (T3), and the brain in the females treated with 72 mg/kg of the Hp extract. The fluorescence observed in the blood, spleen and kidney of the treated mothers was not significantly stronger than the one observed in control mothers (Fig. 1). The analysis of the fetus revealed that the fluorescence intensity was significantly increased in the liver of the fetus obtained from mothers treated

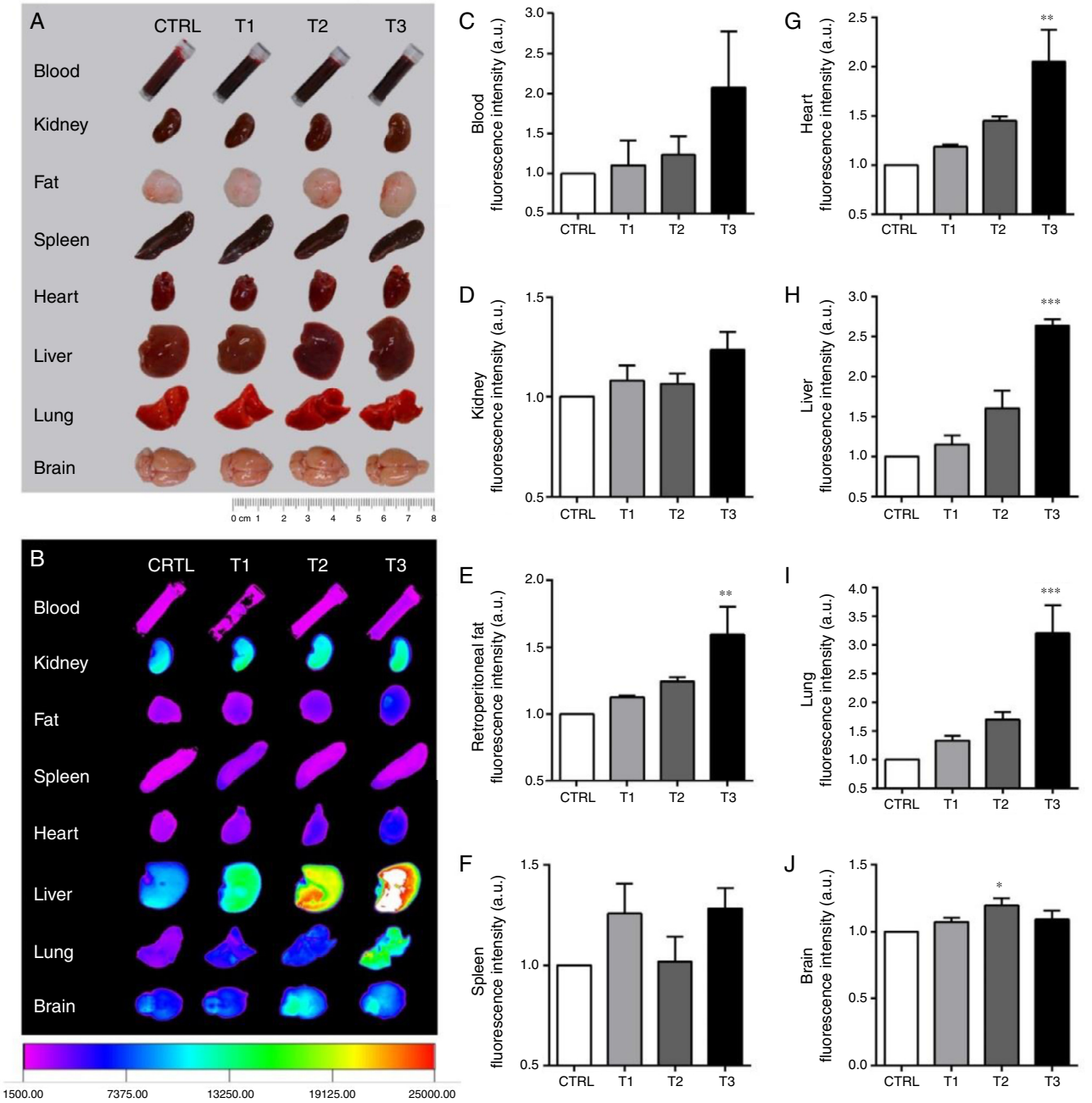

Fig. 1. Fluorescence of hyperic-treated pregnant females. (A) Images of organs and tissues obtained from pregnant females (the scale in cm applies to all samples, except for 2 ml tubes containing blood). (B) Images representing the biodistribution of Hp extract. Quantification of the fluorescence intensity of the samples: (C) Blood, (D) Kidney, (E) Retroperitoneal fat, (F) Spleen, (G) Heart, (H) Liver, (I) Lung, and (J) Brain. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; Values are mean ± S.D.M. ($n = 4$ females per group) (ANOVA test, followed by the Dunnett's test).

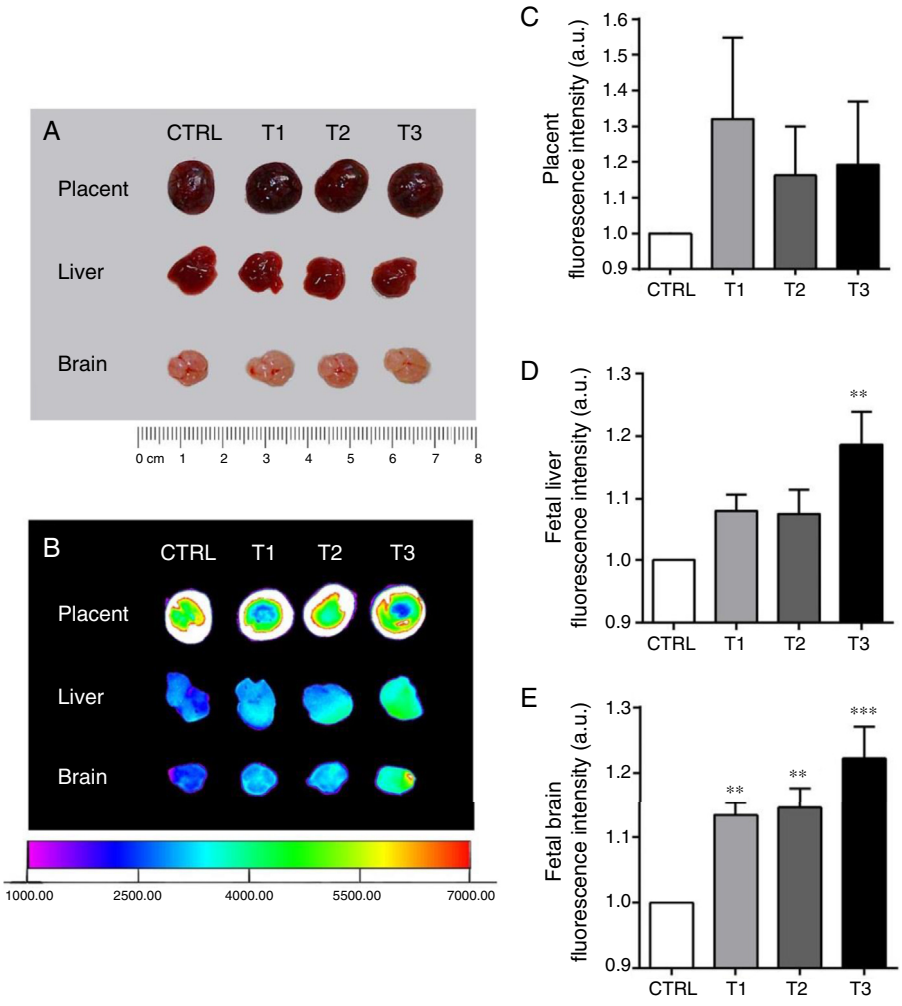

Fig. 2. Fluorescence observed in the fetuses of hyperic-treated pregnant females. (A) Images of organs and tissues obtained from the fetuses. (B) Images representing the biodistribution of Hp extract. Quantification of the fluorescence intensity of the samples: (C) Placenta, (D) Liver and (E) Brain. $^{**}p < 0.01$ and $^{***}p < 0.001$. Values are mean ± S.D.M. ($n = 12$ offspring per group) (ANOVA test, followed by the Dunnett's test).

with 144 mg/kg (T3) and in the brain of the fetus obtained from mothers treated with all three dose levels (T1, T2 and T3), but not at placenta (Fig. 2).

Discussion

The use of any medication that might have toxic effects on the organism should be done with restrictions, especially during gestation. In this context, the use of synthetic or natural antidepressants by pregnant women is a subject of concern since it is not clear yet if there is any relation between the use of antidepressants during pregnancy and the recurrence of postpartum depression. Furthermore, it is also of concern if the use of these medications produces any kind of side effects on the fetal organism and if there are any consequences for postnatal development of the descendents, including adulthood.

In treated animals, body weight loss could be indicating systemic toxicity (Hiremath et al., 1997), although such change, as a result of reduced food intake, could also be indicating a depressive episode (Nepomuceno et al., 2005; Beck and Brad, 2008). In this study, no significant body weight alterations were detected in the adult offspring (90 days old) born from mothers treated with Hp extract during the gestational period. However, substances capable of producing systemic toxicity do not usually display the same degree of toxicity in all organs, but may instead exert toxic effects on one or two target organs, such as the renal and hepatic tissues (Gregoretti et al., 2004).

Hyperic or St John's wort is a well-known natural antidepressant used to treat light and moderate depression (Bahls, 2001; Howland, 2010; Nahas and Sheikh, 2011). Many studies have evidenced the preclinical and clinical efficacy of Hp when compared to the effects of synthetic antidepressants and placebos (Sarris et al., 2011). In rats, a significant action of Hp in the CNS occurred at dose levels ranging from 30 to 100 mg/kg/day, which, taking into account the body surface area, is compared to the clinical dose used in humans (Crupi et al., 2011; Gregoretti et al., 2004). The Hp extract exhibits less adverse effects than the traditional synthetic antidepressants and include phototoxicity, gastrointestinal and allergy problems. However, the common side effects shown by psychoactive substances, such as sedation, memory loss intolerance and addiction, were not reported in patients taking Hp extracts or prescription drugs containing the same constituents (Woelk et al., 1994; Linde et al., 1996; Laakmann et al., 1998; Brattstrom, 2009). Few studies about the use of Hp during pregnancy have been found in the literature. A study developed by Borges and collaborators (2005) indicated that the treatment of pregnant rats did not produce toxic effects to the mother or embryo (Borges et al., 2005). Similar results were obtained in studies with pregnant women and the risk of fetal malformations and prematurity was equivalent to the one expected for the general population (Moretti et al., 2009). However, a study

developed in a rodent model revealed toxic effect of Hp, suggesting the passage of constituents of the extract through the placental barrier, establishing a direct contact with the developing CNS of the fetus (Gregoretti et al., 2004).

In the present study an efficient visualization of Hp extract in some vital organs was obtained by IVIS. The highest used dose (144 mg/kg) presented the most significant fluorescent display in the mothers although in the brain the fluorescence was unexpectedly stronger with the dose of 72 mg/kg. The antidepressant effects (and antinociceptive) Hp extract are given between doses of 30 mg/kg and 100 mg/kg, and a reduction of these activities occur at doses lower than 30 mg/kg and above 100 mg/kg (Isacchi et al., 2009; Galeotti et al., 2010), characterizing the St John's wort-induced antidepressants effects endowed with bell-shaped trend. This decrease in fluorescence indicates that the Hp extract was removed from the brain by transport proteins from blood-brain barrier or was used after connected to the corresponding receptors on target cells. The results show that the binding of the receptor to extract components resulted in effects described in the article. The p-glycoprotein (p-gl) is a carrier protein found in many organs, including brain, organizing the blood-barrier barrier (Ott et al., 2010). The hyperic-extract is metabolized (and can be active and inactivate) by liver enzymes (cytochrome P450) and by p-glycoprotein (Russo et al., 2014). The activity p-gl can be regulated by protein kinase C (PKC)(Ott et al., 2010), than is inactivated by hypericin, an herb component of St. John, through its dephosphorylation (Galeotti and Ghelardini, 2013). Although the explanation for the lower fluorescence found at a dose of 144 mg/kg compared with 72 mg/kg is not complete, it can be assumed that even a dose of 100 mg/kg, different components of extract (hyperforin, hypericin and quercetin) antagonize the excretory effects of p-glycoprotein (Ott et al., 2010). Hypericin and quercetin, for example, inhibit the activation of PKC to reduce the activity of p-gl (Galeotti et al., 2010; Ott et al., 2010); the hyperforin promotes inhibition by a route different from this, probably through direct binding of this glycoprotein (Ott et al., 2010). However, the fluorescence found in dosages exceeding 100 mg/kg can be explained by the hypothesis that there is an increase in PKC expression and/or amount of p-glycoprotein (*up-regulation*) in response to inhibition maintained by the herb components St. John, thereby increasing the efflux of this extract. Since the p- glycoprotein this more active, Hp extract becomes available in the nervous tissue and consequently the greater its effect on it.

In the fetus, the intense fluorescence in the brain at all dose levels indicates the passage of the extract through the placenta and suggests a possible action of the extract on the CNS. A fluorescence emission is also noticeable in the organs of the control group most likely caused by endogenous fluorophores, which generate a native fluorescence, or autofluorescence, when excited with light at a suitable wavelength.

In order to further analyze the consequences of the use of Hp during gestation, this work investigated the possible action in the adult offspring born from mothers treated with the Hp extract during the gestational period by use of various behavioral laboratory-based research protocols (rotarod, pentobarbital-induced sleep time, hole board, elevated plus maze and forced swim test). Because of the concern for impairment in motor behavior from the use of medications, the rotarod test is frequently used to screen-out drugs that might later cause subtle impairments. The length of time spent by a given animal on the rotating rod is a measure of their physical condition, coordination, balance, and motor-planning (Stemmelin et al., 2008). As to the pentobarbital-induced sleep time, this test is a pharmacological model used to investigate sedative-hypnotic drugs and, therefore, was used in this study to assess the depressant activity of Hp extract. The increase in sleep time and the reduction in the time spent on the rotating rod in the offspring born from mothers treated with 144 mg/kg (T3) of the extract suggest that the treatment of mothers with Hp during gestation interferes with the CNS development of the offspring, which is discussed further. The effectiveness of antidepressants was also measured by the forced swim test. The former involves the response of the animal to the threat of drowning and immobility is interpreted as a behavioral correlate of negative mood that leads the animal to a sense of hopelessness. When treated with antidepressants, the animal swims harder and longer than controls (Petit-Demouliere et al., 2005). The offspring born from mothers treated with 144 mg/kg (T3) of the Hp extract showed reduced immobility time in this test in addition to increased latency in the forced swim test, indicating interference in the normal behavior of the animals. The effects observed on the behavioral despair tests do not appear to be influenced by the level of motor impairment detected by rotarod test because no ambulation impairment was found in the hole-board test.

In the anxiety tests, the elevated plus maze test is commonly used to assess rodent anxiety and is based on the animal's aversion of open spaces, involving avoidance of open areas by spending more time in enclosed spaces. Therefore, the administration of anxiolytic substances generally increases the frequency of entries into the open arms and the time spent in them (Grundmann et al., 2007, 2009). The hole-board test also evaluates emotionality and anxiety and is a rodent model test for measuring head-dipping activity which is inversely proportional to the anxiety state of the animals. An increased response could represent the expression of an anxiolytic reaction of the animal (Han et al., 2009). The behavior exhibited by the offspring born from mothers treated with 144 mg/kg (T3) of the extract – increased amount of time spent in the open arms and increased head-dipping and ambulation – suggests that the treatment of mothers with Hp extract during gestation apparently interferes with the anxious behavior of their descendents, evaluated in the adulthood, making them less anxious than control animals.

The obtained results contradict other data found in the literature in which prenatal exposure to Hp did not seem to interfere with the development of the CNS of the offspring (Cada et al., 2001). In this previous work, the medication was given to the animals mixed with the food as opposed to the oral treatment used in this study. In this case, a possible interaction of Hp with other substances used concomitantly should also be taken into account as they could be interfering with the effect of the extract on both the mother's and the offspring's organism (Bahls, 2001).

The exposure to the Hp extract (144 mg/kg) during gestation improved the symptoms of anxiety and depression even after 10 and 60 days post-treatment (Vieira et al., 2013). This demonstrates that alterations in regions of the CNS associated with changes in emotional reactivity are long-lasting (perhaps permanent) and extrapolate the pharmacokinetics properties of the extract, which shows reduction of effects 120 min after administration (Galeotti et al., 2010).

The long-term use of Hp could set adaptations and alterations in the signal transduction, in the activity of intracellular messengers or in the number, expression and regulation of neurotransmitter receptors (Franklin, 2003; Ruedeberg et al., 2010). It could also produce morphological adaptations in the hippocampus nerve cells (Crupi et al., 2011). Such facts added to the hypothesis that Hp can cross the placental barrier could justify the results found in this work in which the use of Hp extract by pregnant mothers indicated reduction of the depressive- and anxious-like state of the descendants.

Therefore, it is suggested that treatment with Hp extract during gestation has generated tolerance in the developing SNC of the fetuses (*down-regulation*) (Ito et al., 1996; Stahl, 1998; Liang et al., 2007). As neurodevelopment continues postnatally with the

increase in the number of neurotransmitter receptors, such as 5-HT (Gaspar et al., 2003), NOR (Murrin et al., 2007) and GABA (Xia and Haddad, 1992), it is believed that a rebound increase in the number of these receptors (*upregulation*) has occurred during this period, which was able to reprogram regions of the brain related to anxiety and depressive behavior (such as the hippocampus) to a new state of normality. This could explain the observed improvement in anxiety and depression episodes and the alterations in memory, learning, and deambulation, which, however, were not in the scope of this work. The characterization of the exact mechanism involved in the generation of tolerance as well as the participation of neurotransmitters and their receptors could be the subject of further studies.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Authors contributions

Participated in research design: RCSS, VAV, VMP and MOG; Conducted experiments: VAV, LVC, LRS and JJ; Contributed new reagents or analytic tools: VMP and MOG; Performed data analysis: RCSS, VAV, LVC and JJasmin; Wrote or contributed to the writing of the manuscript: RCSS and LVC.

Conflicts of interest

The authors declare no conflicts of interest.

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