

*Full Length Research Paper*

## Antidepressant-like effects of two commercially available products of *Hypericum perforatum* in the forced swim test: A long-term study

René Lozano–Hernández<sup>1,2</sup>, Juan Francisco Rodríguez-Landa<sup>1,2\*</sup>, Jesús David Hernández–Figueroa<sup>2</sup>, Margarita Saavedra<sup>1,2</sup>, Fernando Rafael Ramos–Morales<sup>3</sup> and Jesús Samuel Cruz–Sánchez<sup>4</sup>

<sup>1</sup>Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, México.

<sup>2</sup>Facultad de Química Farmacéutica Biológica Zona-Xalapa, Universidad Veracruzana, Xalapa, Veracruz, México.

<sup>3</sup>Unidad de Servicios de Apoyo en Resolución Analítica (SARA), Universidad Veracruzana, Xalapa, Veracruz, México.

<sup>4</sup>Instituto de Ciencias Básicas, Universidad Veracruzana, Xalapa, Veracruz, México.

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Some herbal products of *Hypericum perforatum* (Hypericaceae) are recommended for the treatment of depression. Nevertheless, some of these products do not produce antidepressant-like effects when they are evaluated in experimental models of depression, whereas others remain to be evaluated. Consequently, the antidepressant-like effects of two commercially available products of *H. perforatum* were evaluated and compared with the clinically effective antidepressant fluoxetine. Male Wistar rats received different doses of two products of *H. perforatum* or fluoxetine, and their effects were evaluated at 7, 14, 21, and 28 days of treatment in the open field and forced swim tests. *H. perforatum* products significantly increased the latency to first immobility and reduced total immobility time in the forced swim test, results similar to fluoxetine, without increasing general locomotor activity in the open field test. *H. perforatum* products required 21 days of treatment to exert their antidepressant-like effect, whereas fluoxetine required only 14 days. In conclusion, *H. perforatum* products evaluated in the present study produced an antidepressant-like effect, even at lower doses than those reported previously to be effective in the forced swim test. However, *H. perforatum* required more days of treatment to exert its antidepressant-like effect compared with the antidepressant fluoxetine.

**Key words:** Antidepressant, fluoxetine, forced swim test, Hypericaceae, *Hypericum perforatum*, open field test, rats.

### INTRODUCTION

*Hypericum perforatum* is an herbaceous perennial plant member of the Hypericaceae family. The genus hypericum contain around 400 species divided in 30 subgroups that are spread throughout temperate and tropical areas worldwide. *H. perforatum* is native to the Mediterranean region but has been widely disseminated around the world. *H. perforatum* has been known for a long time for its putative medicinal properties including wound-healing, antibiotic, antiviral and diuretic effects.

Nowadays, phytotherapy based on *H. perforatum* extracts is employed commonly and widely for the treatment of mild forms of depression around the world. Despite controversial data with regard to the antidepressant effects of *H. perforatum*, clinical (Kasper et al., 2008) and preclinical (De Vry et al., 1999; Müller et al., 2001) studies have reported that some extracts from the aerial parts of this plant possess a pharmacological profile similar to clinically effective antidepressants, such as tricyclics and selective serotonin reuptake inhibitors (Rodríguez-Landa and Contreras, 2003). The antidepressant effect is posited to be mainly exerted by two of the major chemical compounds contained in *H. perforatum*

\*Corresponding author. E-mail: [juarodriguez@uv.mx](mailto:juarodriguez@uv.mx).

extracts, hypericin and hyperforin (Butterweck et al., 1997; Chatterjee et al., 1998), with the possible participation of other less abundant chemical compounds, such as flavonoids, biflavonoids, phloroglucinols, naphthodiantrones, xanthenes, proanthocyanidins, acid phenols, essential oils and other phenolic compounds (Barnes et al., 2001; Hostettmann and Wolfender, 2005). In fact, flavonoids have been reported to be necessary for *H. perforatum* extracts to produce their antidepressant-like effects in behavioral models of depression (Calapai et al., 1999; Butterweck et al., 2000; Nöldner and Schötz, 2002; Butterweck and Schmidt, 2007). To date, *H. perforatum* appears to have multiple mechanisms of action involving nonselective serotonin, norepinephrine and dopamine reuptake inhibition and inactivation of monoamine oxidase enzyme activity. Moreover, facilitation of  $\gamma$ -aminobutyric acid (GABA) action on the GABA<sub>A</sub> receptor and activation of sigma-1 receptors have also been shown to be involved (Cott, 1997; Barnes et al., 2001; Mennini and Gobbi, 2004).

Presently, several pharmaceutical companies have elaborated and commercialized *H. perforatum* products, which are recommended for the treatment of mild and moderate depression. Nevertheless, some of these products do not produce antidepressant-like effects when they are evaluated in experimental models of depression. Guilhermano et al. (2004) found that intraperitoneal administration (24, 5, and 1 h before the test) of two available products of *H. perforatum* commercialized in Brazil lack antidepressant-like effects in the forced swim test (FST), an experimental animal model broadly validated for the screening of potential antidepressants (Porsolt et al., 1977). These authors suggested that the ineffectiveness of these two products to produce antidepressant-like effects in the FST could depend on factors that modify the content and quality of the biochemical compounds in the plant, such as cultivation from different geographic regions, the harvesting process, storage, drying, the extraction method, and the herbal product elaboration process (Bilia et al., 2001).

In Mexico, some herbal products and dietary supplements elaborated with standardized dry extract of *H. perforatum* have been commercialized, and they are prescribed for the treatment of mild and moderate depression. In a previous study (Tortoriello et al., 2003), some of these products were reported to have lower concentrations of hypericin and hyperforin than the concentrations reported to produce antidepressant-like effects at the experimental level. In other products, hypericin and hyperforin were not detected (Tortoriello et al., 2003). Consequently, in the present study, the antidepressant-like effects of different doses of two commercially available products of *H. perforatum* (Hiperikan<sup>®</sup> and Remotiv<sup>®</sup>) in Mexico were evaluated in Wistar rats subjected to the OFT and FST. The effects of *H. perforatum* were compared with the minimum effective dose (1.0 mg/kg) of the clinically effective antidepressant fluoxetine reported to produce an antidepressant-like effect

in the FST after long-term treatment (Contreras et al., 2001).

## METHODS

### Animals

Ninety adult male Wistar rats weighing between 250 - 300 g at the beginning of the experiments were used. The rats were housed in Plexiglas cages (six rats per cage), with a 12 h/12 h light/dark cycle (lights on at 06:00 h), an average room temperature of 25°C ( $\pm$  1°C), and *ad libitum* access to water and food. All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Research Council, 1996) and the Norma Oficial Mexicana para el Uso y Cuidado de Animales (NOM-062-ZOO, 1999).

### Drugs and dosage

Two commercially available products elaborated with the standardized dry extract of *H. perforatum* were evaluated: Hiperikan<sup>®</sup> (produced, authenticated and elaborated by Dr. Willmar Schwebel GmbH and Co. KG, Karlsruhe, Germany and distributed by Laboratorios Farmasa S.A. de C.V., México DF, México) and Remotiv<sup>®</sup> (produced, authenticated and elaborated by Max Zeller SÖHNE AG Seeblickstrasse 4, CH Romanshorn, Suiza and distributed by Grünenthal de México, S.A. de C.V., México DF, México). The two products had an equivalent content of 0.50 mg hypericin in 250 mg of dry extract as reported by the manufacturer. Additionally, Tortoriello et al. (2003) in a phytochemical study (HPLC) reported a similar content of hypericin as that reported by the manufacturer in both products of *H. perforatum*, although hyperforin content was different in each product (Hiperikan<sup>®</sup>, 11.3 mg/250 mg of dry extract; Remotiv<sup>®</sup>, 0.20 mg/250 mg of dry extract). To compare the antidepressant-like effects of both products of *H. perforatum*, we utilized the chlorhydrate of fluoxetine (Prozac<sup>®</sup>, authenticated and elaborated by Eli-Lilly Compañía de México, S.A. de C.V., México DF, México), a selective serotonin reuptake inhibitor clinically effective in the treatment of depression.

The doses (mg/kg) of Remotiv<sup>®</sup> and Hiperikan<sup>®</sup> used in the present study were calculated considering the total daily consumption in 24 h recommended by the manufacturer for the treatment of depression: Hiperikan<sup>®</sup> (900 mg), Remotiv<sup>®</sup> (500 mg). The doses were calculated based on a person weighing an average of 70 kg. Thus, the calculated doses were 12.86 mg/kg (D1) of Hiperikan<sup>®</sup> and 7.14 mg/kg (D1) of Remotiv<sup>®</sup> per day. One-quarter (D<sup>1/4</sup>), one-half (D<sup>1/2</sup>), and double (D2) the human dose (D1) were also calculated. Therefore, the evaluated doses of Hiperikan<sup>®</sup> were 3.21 mg/kg (D<sup>1/4</sup>), 6.43 mg/kg (D<sup>1/2</sup>), 12.86 mg/kg (D1), and 25.72 mg/kg (D2), and the calculated doses of Remotiv<sup>®</sup> were 1.78 mg/kg (D<sup>1/4</sup>), 3.57 mg/kg (D<sup>1/2</sup>), 7.14 mg/kg (D1), and 14.28 mg/kg (D2), every 24 h. We calculated doses in mg/kg (but not in mg/rat) to avoid possible differences in pharmacological actions associated with changes in rat weight such that the doses were adjusted daily to the body weight of the rat during the study.

Generally, fluoxetine doses reported to have antidepressant-like effects in the FST are 5 - 20 mg/kg with an acute schedule of treatment (Page et al., 1999; Rogóz et al., 2008). Long-term treatment (21 days) has been reported to require 1.0 mg/kg every 24 h to produce antidepressant-like effects in wistar rats (Contreras et al., 2001). Therefore, considering the long-term treatment used in the present study, the dose of 1.0 mg/kg every 24 h was selected.

All treatments were administered once per day for 28 days (10:00 h) at a volume equivalent to 1.0 ml/kg of purified water through a displaceable sterilized intraesophageal polyethylene cannula (S-54-

–HL Cole-Parmer Co, Vernon Hills, IL, USA: 4 cm length  $\times$  1.0 mm diameter), coupled to a 5 ml disposable syringe (B–D Plastipak, Becton Dickinson and Co, Cuautitlán Izcalli, Estado de México, México). The behavioral tests were performed between 11:00 h and 13:00 h.

### Experimental groups

The rats were assigned to 10 independent groups ( $n = 9$  per group). The control group received the vehicle in which fluoxetine or the products of *H. perforatum* were dissolved (purified water). The fluoxetine group received 1.0 mg/kg of fluoxetine chlorhydrate. Another four groups received different doses of Hiperikan<sup>®</sup> (3.21, 6.43, 12.86 and 25.72 mg/kg), and the last four groups received different doses of Remotiv<sup>®</sup> (1.78, 3.57, 7.14 and 14.28 mg/kg).

Before initiating the administration of pharmacological treatments, all rats were subjected to a 5 min pretest in the OFT and a 15 min pretest in the FST, the results of which were discarded from the statistical analysis. In the FST pretest, the rats confronted a new emergency situation represented by swimming. This allowed us to assure the development of behavioral “despair” in the rat. Twenty-four hours later (defined as day 0), rats were subjected to a 5 min test session in the OFT and FST to evaluate baseline behavioral activity. After this test session, the pharmacological treatments were initiated, the effects of which were evaluated on the 7th, 14th, 21st, and 28th day of treatment, 1 h after the corresponding administration on the test day. All experimental sessions were videotaped, and two independent observers blind to treatment measured the behavioral variables.

### Behavioral tests

#### Open field test

To evaluate the effect of the drugs on spontaneous locomotor activity, rats were individually placed in an opaque Plexiglas cage (44  $\times$  33 cm) with walls 20 cm high and the floor divided into 12 squares (11  $\times$  11 cm). When the hind legs crossed the line of the squares, the rat was considered to have crossed from one square to another (crossing). After each rat was tested in the OFT, the cage was carefully cleaned with a solution containing 0.5% ammonia, 15% ethanol, 10% extran, 5% isopropanol, 10% pinol and 59.5% purified water to remove the scent of the previously evaluated rat, which could modify the spontaneous behavior of the rat (Gutiérrez-García et al., 2006). After the OFT, the rat was immediately subjected to the FST.

#### Forced swim test

In this test, the rats were individually forced to swim in a rectangular pool (50  $\times$  30  $\times$  60 cm) with 24 cm deep water ( $25 \pm 1^\circ\text{C}$ ). The evaluated variables were the following: latency to first immobility and total immobility time (i.e., when the rat floated without making vigorous movements leading to displacements and only maintained its head above the water surface for more than 2 s).

### Statistical analysis

Data from the OFT and FST were analyzed using two-way repeated measures analysis of variance (ANOVA) because the same rats were subjected to several test sessions. Treatments (vehicle, fluoxetine and the doses of products of *H. perforatum*) were the first factor and treatment days (0, 7, 14, 21, and 28 days) were the

second factor. Student-Newman-Keuls *post hoc* test was applied when  $p$  values in the ANOVA were  $\leq 0.05$ . Results are expressed as mean  $\pm$  standard error.

## RESULTS

### Open field test

The analysis of crossings in the OFT revealed significant differences by treatment ( $F_{9,320} = 6.4$ ,  $p < 0.001$ ) and days of treatment ( $F_{4,320} = 56.2$ ,  $p < 0.001$ ) and a significant treatment  $\times$  days interaction ( $F_{36,320} = 3.7$ ,  $p < 0.001$ ). The *post hoc* test revealed that crossings were significantly ( $p < 0.05$ ) reduced from day 14 of treatment in the fluoxetine group compared with day 0 and compared with day 14 in the control group. The D $\frac{1}{2}$  dose of Remotiv<sup>®</sup> also significantly ( $p < 0.05$ ) reduced crossings from day 21 of treatment and the D2 dose of Hiperikan<sup>®</sup> reduced crossings from day 28 of treatment compared with day 0 and the respective session of the control group. Crossings remained unchanged throughout the study in the control group (Table 1).

### Forced swim test

#### Latency to first immobility

The analysis of the latency to first immobility revealed significant differences by treatment ( $F_{9,320} = 72.1$ ,  $p < 0.001$ ) and days of treatment ( $F_{4,320} = 202.5$ ,  $p < 0.001$ ) and a significant treatment  $\times$  days interaction ( $F_{36,320} = 24.2$ ,  $p < 0.001$ ). The *post hoc* test revealed that rats treated with fluoxetine significantly increased the latency to first immobility from day 14 of treatment compared with day 0 and compared with day 14 of the control group. The rats treated with the D1 and D2 doses of Remotiv<sup>®</sup> and D $\frac{1}{2}$ , D1 and D2 doses of Hiperikan<sup>®</sup> significantly increased the latency to first immobility from day 21 compared with day 0 and the respective session of the control group. The control group had a shorter latency to first immobility on day 21 compared with day 0 (Table 2).

#### Total immobility time

The analysis of total immobility time revealed significant differences by treatment ( $F_{9,320} = 27.6$ ,  $p < 0.001$ ) and days of treatment ( $F_{4,320} = 30.9$ ,  $p < 0.001$ ) and a significant treatment  $\times$  days interaction ( $F_{36,320} = 12.6$ ,  $p < 0.001$ ). The *post hoc* test revealed that rats treated with fluoxetine reduced total immobility time from day 14 of treatment compared with day 0 and compared with day 14 of the control group. Total immobility time in rats treated with the D1 and D2 doses of Remotiv<sup>®</sup> and D $\frac{1}{2}$ , D1, and D2 doses of Hiperikan<sup>®</sup> was significantly reduced on day 21 compared with day 0 and compared with day 21 of the control group (Table 3).

**Table 1.** Effect of vehicle, fluoxetine, Hiperikan<sup>®</sup>, and Remotiv<sup>®</sup> on the number of crossings in the OFT in 5 min.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
<b>Ctrl (vehicle)</b>	35.3 ± 2.8	32.8 ± 1.6	31.2 ± 1.3	30.5 ± 1.8	27.7 ± 1.6
<b>Fluoxetine mg/kg</b>					
1.0	37.6 ± 2.2	31.0 ± 1.8	17.4 ± 0.8 *+	11.7 ± 1.3 *+	10.1 ± 1.3 *+
<b>Remotiv<sup>®</sup> (mg/kg)</b>					
D¼ (1.78)	31.3 ± 1.5	24.6 ± 3.6	32.2 ± 4.8	24.4 ± 2.9	24.5 ± 3.7
D½ (3.57)	37.2 ± 4.5	25.7 ± 3.3	29.2 ± 2.3	11.2 ± 1.4 *+	10.1 ± 1.3 *+
D1 (7.14)	34.1 ± 4.3	30.4 ± 1.7	29.8 ± 1.9	19.7 ± 2.4	23.1 ± 2.1
D2 (14.28)	24.4 ± 2.8	17.1 ± 1.8	20.2 ± 2.6	12.5 ± 1.2	18.9 ± 3.3
<b>Hiperikan<sup>®</sup> (mg/kg)</b>					
D¼ (3.21)	58.3 ± 11.9	27.0 ± 3.8	16.2 ± 3.5	22.0 ± 3.0	36.0 ± 3.6
D½ (6.43)	38.3 ± 2.7	32.7 ± 2.3	28.6 ± 2.4	16.1 ± 1.4	21.8 ± 0.9
D1 (12.86)	40.0 ± 1.2	29.7 ± 1.3	31.0 ± 1.6	21.5 ± 1.2	18.3 ± 1.1 *+
D2 (27.71)	50.1 ± 7.1	34.0 ± 5.2	40.0 ± 8.7	27.1 ± 3.7	30.3 ± 5.3

Values are expressed as mean ± standard error from nine rats. \*p < 0.05, compared with day 0 of the same group; \*p < 0.05, compared with respective session of control group (two-way repeated-measures ANOVA followed by Student Newman-Keuls *post hoc* test).

**Table 2.** Effect of vehicle, fluoxetine, Hiperikan<sup>®</sup>, and Remotiv<sup>®</sup> on latency to first immobility (s) in the FST in 5 min.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
<b>Ctrl (vehicle)</b>	55.5 ± 4.2	37.8 ± 3.2	36.3 ± 2.4	17.5 ± 1.7 *	16.5 ± 2.4 *
<b>Fluoxetine (mg/kg)</b>					
1.0	54.0 ± 3.4	41.0 ± 2.7	94.6 ± 4.8 *+	123.9 ± 8.4 *+	132.8 ± 11.3 *+
<b>Remotiv<sup>®</sup> (mg/kg)</b>					
D¼ (1.78)	31.7 ± 3.6	20.3 ± 2.7	34.3 ± 3.9	20.4 ± 3.5	25.4 ± 3.0
D½ (3.57)	34.4 ± 3.5	14.6 ± 1.4	27.9 ± 4.5	39.5 ± 4.3	26.4 ± 3.1
D1 (7.14)	35.7 ± 3.4	29.2 ± 2.3	42.9 ± 4.8	115.6 ± 6.6 *+	128.7 ± 8.4 *+
D2 (14.28)	24.8 ± 1.5	16.7 ± 1.4	43.5 ± 9.1	132.5 ± 9.7 *+	131.7 ± 9.7 *+
<b>Hiperikan<sup>®</sup> (mg/kg)</b>					
D¼ (3.21)	28.7 ± 2.9	22.1 ± 3.5	30.8 ± 2.1	16.3 ± 2.1	19.5 ± 3.2
D½ (6.43)	56.5 ± 4.6	42.1 ± 3.5	53.8 ± 2.8	111.6 ± 10.6 *+	124.7 ± 9.4 *+
D1 (12.86)	46.3 ± 3.3	22.5 ± 1.5	55.4 ± 8.9	108.5 ± 8.4 *+	113.2 ± 9.3 *+
D2 (27.71)	37.1 ± 4.5	31.5 ± 1.5	65.5 ± 8.9	128.9 ± 11.4 *+	116.3 ± 8.6 *+

Values are expressed as mean ± standard error from nine rats. \*p < 0.05, compared with day 0 of the same group; \*p < 0.05, compared with respective session of control group (two-way repeated-measures ANOVA followed by Student-Newman-Keuls *post hoc* test).

## DISCUSSION

The FST is a behavioral test validated for the screening of substances with potential antidepressant activity, in which rats develop a state of “despair” characterized by increased immobility (Porsolt et al., 1977). Clinically

effective antidepressants, such as fluoxetine, imipramine and desmethylimipramine (Porsolt et al., 1977; Contreras et al., 2001), reduce immobility and some (e.g., fluoxetine) also increase the latency to first immobility in the FST (Contreras et al., 2001), which are considered antidepressant-like effects at the preclinical level (Porsolt

**Table 3.** Effect of vehicle, fluoxetine, Hiperikan<sup>®</sup>, and Remotiv<sup>®</sup> on total immobility time (s) in the FST in 5 min.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
<b>Ctrl (vehicle)</b>	53.6 ± 3.6	44.3 ± 2.5	53.1 ± 2.0	75.1 ± 10.2	74.0 ± 3.6
<b>Fluoxetine (mg/kg)</b>					
1.0	53.5 ± 4.2	42.4 ± 2.0	26.7 ± 1.7 *+	11.6 ± 1.2 *+	12.7 ± 1.4 *+
<b>Remotiv<sup>®</sup> (mg/kg)</b>					
D <sup>1</sup> / <sub>4</sub> (1.78)	41.1 ± 5.1	63.2 ± 2.1	35.1 ± 2.2	85.8 ± 12.7	73.8 ± 6.5
D <sup>1</sup> / <sub>2</sub> (3.57)	62.8 ± 6.4	64.8 ± 8.6	43.3 ± 4.3	75.6 ± 10.4	76.6 ± 8.3
D1 (7.14)	65.2 ± 4.8	68.7 ± 4.5	55.2 ± 4.7	24.4 ± 3.4 *+	23.3 ± 2.6 *+
D2 (14.28)	66.7 ± 7.9	53.5 ± 4.5	43.8 ± 3.3	18.2 ± 2.1 *+	14.3 ± 1.3 *+
<b>Hiperikan<sup>®</sup> (mg/kg)</b>					
D <sup>1</sup> / <sub>4</sub> (3.21)	65.8 ± 10.2	66.5 ± 5.5	59.1 ± 6.9	74.2 ± 3.4	77.8 ± 5.7
D <sup>1</sup> / <sub>2</sub> (6.43)	65.1 ± 3.6	55.1 ± 3.2	45.4 ± 1.7	26.2 ± 2.3 *+	31.0 ± 1.8 *+
D1 (12.86)	56.0 ± 2.7	61.0 ± 2.6	37.5 ± 2.9	17.8 ± 1.5 *+	23.0 ± 0.8 *+
D2 (27.71)	69.2 ± 6.2	58.2 ± 5.6	51.6 ± 3.0	16.9 ± 3.9 *+	12.6 ± 1.0 *+

Values are expressed as mean ± standard error from nine rats. \* $p < 0.05$ , compared with day 0 of the same group; + $p < 0.05$ , compared with respective session of control group (two-way repeated-measures ANOVA followed by Student-Newman-Keuls *post hoc* test).

et al., 1977; Wieland and Lucki, 1990; Espejo and Miñano, 1999; Contreras et al., 2000; Rodríguez-Landa et al., 2007, 2009). In the present study, the D1 and D2 doses of Remotiv<sup>®</sup> and D<sup>1</sup>/<sub>2</sub>, D1, and D2 doses of Hiperikan<sup>®</sup> reduced total immobility time and increased the latency to first immobility in the FST, similar to fluoxetine, indicating the antidepressant-like effects of both products elaborated with *H. perforatum*.

An increase in locomotor activity in the OFT may disguise the motivational effects produced by antidepressants, similar to CNS stimulant drugs, that reduce immobility in the FST but increase general locomotor activity (Porsolt et al., 1977; Wieland and Lucki, 1990). In present study, reduced immobility produced by *H. perforatum* products or fluoxetine was not related to any locomotor stimulant action. Some doses of *H. perforatum* products and fluoxetine, in fact, decreased crossings in the OFT. This finding is consistent with previous studies showing that reduced immobility produced by antidepressant drugs or *H. perforatum* extracts is not associated with changes in locomotor activity in the OFT (Wieland and Lucki 1990; De Vry et al., 1999; Butterweck et al., 2000; Panocka et al., 2000; Contreras et al., 2001; Cervo et al., 2002).

Both products of *H. perforatum* produced antidepressant-like effects after 21 days of treatment at doses lower than those reported to be effective in the FST, suggesting that administering higher doses of *H. perforatum* is not necessary to exert these effects in the rat. However, a longer treatment time could be required

so that neurochemical changes in the brain are established to produce their antidepressant-like effects, similar to clinically effective antidepressant drugs (López-Rubalcava and Lucki, 2000; Contreras et al., 2001; Rodríguez-Landa et al., 2003).

Additionally, based on a previous phytochemical study of both products of *H. perforatum* (Tortoriello et al., 2003), in the present study the minimum effective doses of Remotiv<sup>®</sup> (D1 hypericin, 0.0117 mg/kg; D1 hyperforin, 0.0045 mg/kg) and Hiperikan<sup>®</sup> (D<sup>1</sup>/<sub>2</sub> hypericin, 0.0137 mg/kg; D<sup>1</sup>/<sub>2</sub> hyperforin, 0.0042 mg/kg) with antidepressant-like effects were lower than the minimum effective doses of hypericin (0.23 mg/kg, single dose) and hyperforin (0.19 mg/kg, administered 23.5, 5 and 0.5 h before the test) reported to have antidepressant-like effects in the FST (Cervo et al., 2002). This indicates that the antidepressant-like effects of Remotiv<sup>®</sup> and Hiperikan<sup>®</sup> may not only be associated with the content of these two chemical compounds (hypericin and hyperforin), but possibly also other constituents of the plant, such as procyanidins, pseudohypericin and principal flavonoids, as suggested previously by some authors (Butterweck et al., 1997, 2000; Hostettmann and Wolfender, 2005).

The differences in the required time of treatment between the products of *H. perforatum* and fluoxetine to produce antidepressant-like effects may be attributed to the nonselective pharmacological actions in the brain and noncompetitive actions on aminergic molecular transporters produced by *H. perforatum* chemical compounds

(Müller et al., 2001; Hirano et al., 2004), whereas fluoxetine, as with the majority of selective serotonin reuptake inhibitors, selectively competes with these transporters in the brain (Hirano et al., 2004). Such a mechanism possibly influences the manifestation of these antidepressant-like effects of fluoxetine compared with *H. perforatum* products.

Finally, in the present study, long-term treatment with lower doses (6.43 - 27.71 mg/kg) of *H. perforatum* products than those reported to be effective in the FST after acute treatment 450 - 900 mg/kg (Butterweck et al., 1997; Gambarana and Giachetti, 2005) produced antidepressant-like effects in Wistar rats in the FST. These results indicate that higher doses are not required to produce antidepressant-like effects in the rat. In a manner similar to conventional antidepressant drugs, the *H. perforatum* products require 2 to 3 weeks of treatment to exert these effects. The present results may be relevant to future preclinical and clinical studies to explore the possibility of reducing or avoiding potential side effects, such as nausea, rash, fatigue, restlessness, photosensitivity, acute neuropathy and even episodes of mania and pharmacological interactions associated with the highest doses of some *H. perforatum* extracts in humans (for review, see Rodríguez-Landa and Contreras, 2003).

## Conclusion

In the present study, after long-term treatment, Remotiv® and Hiperikan® produced antidepressant-like effects in rats in the FST, even at doses lower than those reported to be effective at reducing immobility in the FST after acute treatment. However, both products of *H. perforatum* required more days of treatment (21 days) to exert their antidepressant-like effects compared with the minimum effective dose of fluoxetine reported to have antidepressant-like effects in the FST after long-term treatment, which required only 14 days of treatment.

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