Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 74 (2003) 1015–1024
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of *Hypericum perforatum* and paroxetine in the mouse defense test battery

Vanessa Beijamini, Roberto Andreatini*

Departamento de Farmacologia, Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Centro Politécnico—Setor de Ciências Biológicas, Universidade Federal do Paraná, C.P. 19031, CEP: 81531-990, Curitiba, PR, Brazil

Received 19 June 2002; received in revised form 20 November 2002; accepted 22 January 2003

Abstract

Since (a) *Hypericum perforatum* shows anxiolytic-like effect in some animal models, (b) antidepressant drugs (AD) have been used as the main drug treatment for panic disorder (PD), (c) AD are also effective in generalized anxiety disorder (GAD), and (d) *H. perforatum* exhibits antidepressant activity, it was hypothesized that *H. perforatum* might possess an antipanic-like and/or anxiolytic-like effect. Previous studies with the mouse defense test battery (MDTB) have suggested that this model may be useful for the investigation of anxiolytic-like and antipanic-like compounds. Thus, the aim of the present study was to evaluate the effect of *H. perforatum* extract in the MDTB. The effect of acute, subchronic (7 days), and chronic (21 days) *H. perforatum* (150 and 300 mg/kg) extract administration was evaluated in mice submitted to the MDTB. Paroxetine (5 mg/kg), a selective serotonin re-uptake inhibitor with anxiolytic and antipanic effect, was used as a positive control. The results showed that 21 days of repeated administration of *H. perforatum* 300 mg/kg and paroxetine 5 mg/kg reduced flight reactions (number of avoidances, avoidance distance, and overall flight speed) to the presence of the predator. While the effect of paroxetine confirms that MDTB is useful for the detection of antipanic-like drugs, the effect of *H. perforatum* suggests a putative antipanic-like effect for this extract. Moreover, after 21 days of repeated administration, paroxetine increased the number of approaches/withdrawals and reduced the number of upright postures, suggesting a partial anxiolytic-like effect, while *H. perforatum* only reduced the number of upright postures. The present results suggest anxiolytic-like and antipanic-like effects of *H. perforatum* extract. However, it should be emphasized that the risk assessment (the main index of anxiety) was not affected by the extract, while the attack reactions were only weakly modified.

Keywords: Animal model; Anxiety; *Hypericum perforatum*; MDTB; Panic; Paroxetine

1. Introduction

Hypericum perforatum L., Hypericaceae, also known as St. John's Wort, is a perennial herbaceous plant native to Europe, Asia, North Africa, and North America (Di Carlo et al., 2001). *H. perforatum* contains numerous compounds with biological activity, such as napthdianthrones, including hypericin and pseudohypericin, tannins and proanthocyanidins, flavonoids, and phloroglucinol derivatives (hyperforin). Clinical studies have suggested that the *H. perforatum* extract is as effective as traditional antidepressants and has superior efficacy compared to placebo for the treatment of mild to moderate depression (for reviews, see Kim et al., 1999; Kasper, 2001; Linde and Mulrow, 2000; Di Carlo et al., 2001; also see Shelton et al., 2001). The clinical evidence has been supported by many pre-clinical studies in animal models of depression (Gambarana et al., 1999; Butterweck et al., 1997; Bhattacharya et al., 1998; Chatterjee et al., 1998a). Regarding its mechanism of action, recent studies have led to propose that hyperforin could be a major neuroactive constituent of the herb (Chatterjee et al., 1998b; Di Carlo et al., 2001). However, unlike conventional antidepressants, this constituent is not a specific reuptake inhibitor, but in fact is able to inhibit synaptosomal uptake of monoamines, glutamate, and γ-aminobutyric acid (GABA) with almost equal potencies (Muller et al., 1998; Wonnemann et al., 2001). In addiction, chronic treatment with *H. perforatum* extract leads to adaptive changes in β-adrenoceptors in the frontal cortex (Muller et al., 1997) like those induced by conventional antidepressant drugs (AD).

* Corresponding author. Tel.: +55-41-361-1716; fax: +55-41-226-2042, +55-41-336-5962.
E-mail address: randreatini@ufpr.br (R. Andreatini).

0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00034-0

Some studies have shown that *H. perforatum* also may exert an anxiolytic effect. In clinical studies, *H. perforatum* extract exerted a beneficial effect on patients with obsessive–compulsive disorder in an open study (Taylor and Kobak, 2000) and reduced anxiety and depression scores in the Hospital Anxiety and Depression Scale in patients with fatigue of unexplained origin (Stevinson et al., 1998). Moreover, there is a case report showing that *H. perforatum* treatment reduced the number of panic attacks in a patient with a possible panic disorder (PD) diagnosis (Yager et al., 1999), and three case reports suggest an anxiolytic effect of the extract (Davison and Connor, 2001).

Considering pre-clinical studies, some recent data suggest a putative anxiolytic-like effect of *H. perforatum*. Acute treatment impaired the acquisition of inhibitory avoidance in the light–dark test in rats (Vandenbogaerde et al., 2000), an effect that was blocked by pretreatment with the benzodiazepine receptor antagonist flumazenil, and decreased the inhibitory avoidance latency of rats submitted to the elevated T-maze (Flausino et al., 2002). In this line of research, our group observed that acute treatment with *H. perforatum* extract decreased the marble-burying behavior of mice at a dose that did not change locomotor activity (Skalisz et al., unpublished data). Acute *H. perforatum* administration to mice increased the time spent in open areas, the percentage of unprotected head-dips, and unprotected stretch approach posture in the elevated plus-maze (Coleta et al., 2001). Chronic treatment with the extract increased the time spent in the light compartment in the light/dark test (Flausino et al., 2002). The ethanolic extract of Indian *H. perforatum* also induced an anxiolytic-like effect on the elevated plus-maze, open-field test and social interaction test after repeated administration for 3 days (Kumar et al., 2000). Our group also showed that repeated treatment (7 days) with *H. perforatum* and paroxetine increased the escape latency of rats submitted to the elevated T-maze in a modified procedure (Beijamini and Andreatini, unpublished data), suggesting an antipanic-like effect, although Flausino et al. (2002) did not show this effect on the elevated T-maze after chronic treatment (14 days).

When considering preclinical data, it is important to keep in mind that pathological anxiety is a heterogeneous phenomenon comprised of generalized anxiety disorder (GAD), PD, obsessive–compulsive disorder (OCD), and phobias (American Psychiatric Association, 1994). PD is a chronic mental disease and its central pathologic feature is the panic attack, which involves the sudden onset of intense apprehension, fearfulness, fear of dying, paresthesias, sweating, dizziness, and palpitations (American Psychiatric Association, 1994). It is clear that many of the behavioral symptoms, which often rely on verbal report, can hardly be modeled in animals. However, it has been suggested that panic may result when 'flight or fight' mechanisms are strongly aroused but no perceived route for escape is available (Deakin and Graeff, 1991). Based on this assumption, several attempts to develop animal paradigms involving flight or escape behaviors have been made, which have been suggested to relate to certain aspects of a panic attack. For instance, Griebel et al. (1995a,b) have developed an animal model called the Mouse Defense Test Battery (MDTB), in which mice are confronted with immediate, discrete, or potential threat stimuli (a rat). This model is based on an ethological validation of natural defensive behaviors of laboratory and wild mice in both semi-natural and highly structured situations (Blanchard et al., 1993a,b, 1997, 1998, 2001; Griebel et al., 1996a,c). Extensive pharmacological evaluation of the MDTB has demonstrated that panic-modulating agents specifically affect animals' flight responses, and anxiolytic drugs (i.e., drugs that are effective in GAD) reduce risk assessment activities and defensive threat/attack responses (Blanchard et al., 1997). Clinically effective antipanic drugs (clonazepam, alprazolam, imipramine, fluoxetine, phenelzine, and moclobemine) decrease flight behaviors (Griebel et al., 1995a,b,d, 1996a,b, 1997a, 1998). On the other hand, drugs that induce panic attacks (yohimbine, flumazenil, and cocaine) or worsen the patient's condition early in treatment of PD (acute fluoxetine and imipramine) increase the flight response (Griebel et al., 1995a,b, 1996a; Blanchard et al., 1997, 1999). Furthermore, ineffective antipanic drugs (chlordiazepoxide, diazepam, 5-HT1A agonists, and 5-HT2 antagonists) do not modify these behaviors (Griebel et al., 1995c). In contrast, risk assessment activities and defensive threat/attack responses are reduced by benzodiazepines (chlordiazepoxide, diazepam, clonazepam, clobazam, and alprazolam), by a 5-HT1A agonist (gepirone), and by chronic treatment with antidepressants (imipramine, fluoxetine and phenelzine) (Griebel et al., 1995a,b, 1996a,b, 1997b, 1998, 1999a). Moreover, flumazenil and RO 194603, respectively a benzodiazepine antagonist and a benzodiazepine inverse agonist, enhance these behaviors (Griebel et al., 1995b).

Because (a) *H. perforatum* shows an anxiolytic-like effect in some animal models, (b) AD have been used as the main drug treatment for PD, (c) AD are also effective in GAD, and (d) *H. perforatum* exhibits antidepressant activity, it was hypothesized that *H. perforatum* might possess antipanic-like and/or anxiolytic-like effect. Thus, the aim of the present study was to evaluate the effect of acute, subchronic (7 days), and chronic (21 days) treatment with *H. perforatum* extract in the MDTB at a dose that exhibited an antidepressant-like effect.

2. Methods

2.1. Animals

The subjects were adult male albino Swiss mice (30–45 g) from the TECPAR (Brazil) breed and adult male albino Wistar rats (250–300 g) from our own breed. They were housed in groups of five in polypropylene cages with

wood shavings as bedding under controlled conditions of light (12-h light–dark cycle, light on at 0700 h) and temperature (22 ± 1 °C). Tap water and food pellets were available ad libitum throughout the experiments. The mice were acclimated to our laboratory housing conditions at least 1 week before the experiments. None of animals (rats and mice) were subjected to handling prior to the beginning of the experiments. The mice were divided into groups of 14–17 animals.

2.2. Drugs

The *H. perforatum* dry standardized extract (LI 160) was supplied by Eurofarma (obtained from Indena, Milan, Italy; extraction solvent: methanol; herb–extract ratio 7:1). The amount of hypericin (0.3%) and hyperforin (3.3%) was quantified by high-performance liquid chromatography and fluorescence detection by Indena. The *H. perforatum* extract was suspended in distilled water and sonicated for 20 min before oral administration. Paroxetine (Eurofarma, São Paulo, Brazil) was dissolved in distilled water. Both drugs were prepared on the day of the experiment. The control group received physiological saline. All drugs were administered orally by gavage in a final volume of 10 ml/kg body weight.

The rats used in the experiments were deeply anaesthetized with an injection of thiopental (Cristalia, São Paulo, Brazil) 25 mg/kg 15 min before the test.

2.3. Apparatus

The MDTB was conducted in an oval runway made of black painted wood (0.4 m wide, 0.3 m high and 4.8 m in total length) consisting of two 2-m straight segments joined to two 0.4-m curved segments and separated by a middle wall (2.0 × 0.3 × 0.06 m). The floor was marked every 20 cm to facilitate distance measurement. The apparatus was elevated to a height of 0.7 m from the floor to enable the experimenter to hold the rat easily, while minimizing the mouse's visual contact with the experimenter. The experiments were recorded with a video camera mounted above the apparatus and were performed between 1300 and 1800 h.

2.4. Procedures

2.4.1. MDTB

The procedure was carried out as proposed by Griebel et al. (1995b).

2.4.1.1. Pretest or locomotor activity before exposure to the predator. The mouse was placed in the center of the runway for 3 min, and the number of line crossings, wall rears, wall climbs, and jump escapes were recorded. The last three measures provide an index of contextual escape attempts.

2.4.1.2. The rat avoidance test. Immediately after the pretest, a deeply anesthetized handheld rat was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the mouse was made or the mouse ran away from the approaching rat. If the subject fled, the number of avoidances and the avoidance distance (the distance between the rat and the mouse at the point of flight in centimeters) were recorded. This was repeated five times. The interval between trials was 15 s.

2.4.1.3. The chase/flight test. The handheld rat was brought up to the mouse at a speed of approximately 2.0 m/s for a distance of 18 m. During the chase, number of stops (pause in movement), orientations (subject stops, then orients the head toward the rat), and reversals (subject stops, then runs in the opposite direction) were recorded. Overall flight speed (m/s) and maximum flight speed (an average of three measures of uninterrupted straight flight, over 1 m linear segment of the runway) were calculated.

2.4.1.4. The straight alley test. Thirty seconds after the chase/flight test, the runway was converted to a straight alley (a segment of 1 m) by closing two doors. Three confrontations at 0.4 m, 15 s each, were made with a handheld rat toward the subject in this inescapable straight alley. Measurements made included immobility time, closest distance between the mouse and the rat, and number of approaches/withdrawals.

2.4.1.5. The forced contact test. The experimenter brought the rat in contact with the subject three times, for 10 s each time. For each contact, the following defensive threat and attack reactions were noted: vocalizations, bites, upright postures, and jump attacks.

2.4.1.6. The post-test or contextual defense test. Finally, the doors and predator were removed. Line crossings and escape attempts were recorded during a 3-min session.

2.5. Treatment schedule

H. perforatum doses were chosen based on its antidepressant-like effect on the forced swimming test (Skalisz et al., unpublished data). In the acute experiment, saline, paroxetine (Paro 5 mg/kg, po, positive control) or *H. perforatum* extract (Hp 150 or 300 mg/kg, po) was administered 1 h before the test session. For the subchronic study, animals were treated with saline, Paro 5 mg/kg or Hp 150 or 300 mg/kg for 7 days once a day (po). On the 7th day, mice were tested in the oval runway 1 h after the last gavage treatment. For the chronic study, the same groups (but consisting of different animals) were treated once a day for 21 days (po). On the 21st day, mice were tested in the oval runway 1 h after the last gavage treatment.

2.6. Statistical analysis

Parametric data are reported as mean ± standard error of mean (S.E.M.) and nonparametric data are reported as median ± semi-inter-quartile range (S.I.R.). Line crossing data before and after confrontation with the rat were analyzed by two-way analysis of variance (ANOVA) followed by the Newman–Keuls post hoc test when appropriate. Overall and maximum speed and immobility time were analyzed by one-way ANOVA followed by the Newman–Keuls post hoc test when appropriate. Nonparametric tests were used for data that did not show homoscedasticity or did not fit the parametric assumptions. Comparisons between treatment groups and control were made by Kruskal–Wallis ANOVA followed by the Dunn test when appropriate. The Wilcoxon matched pair test was used to compare pre- versus post-exposure data for the same group. Differences were considered statistically significant when $P \leq .05$. The statistical analysis was performed using the following software: Statistic 5.5 (Statsoft, 1999) and GraphPad Prism 3.0 (GraphPad Software, 1999).

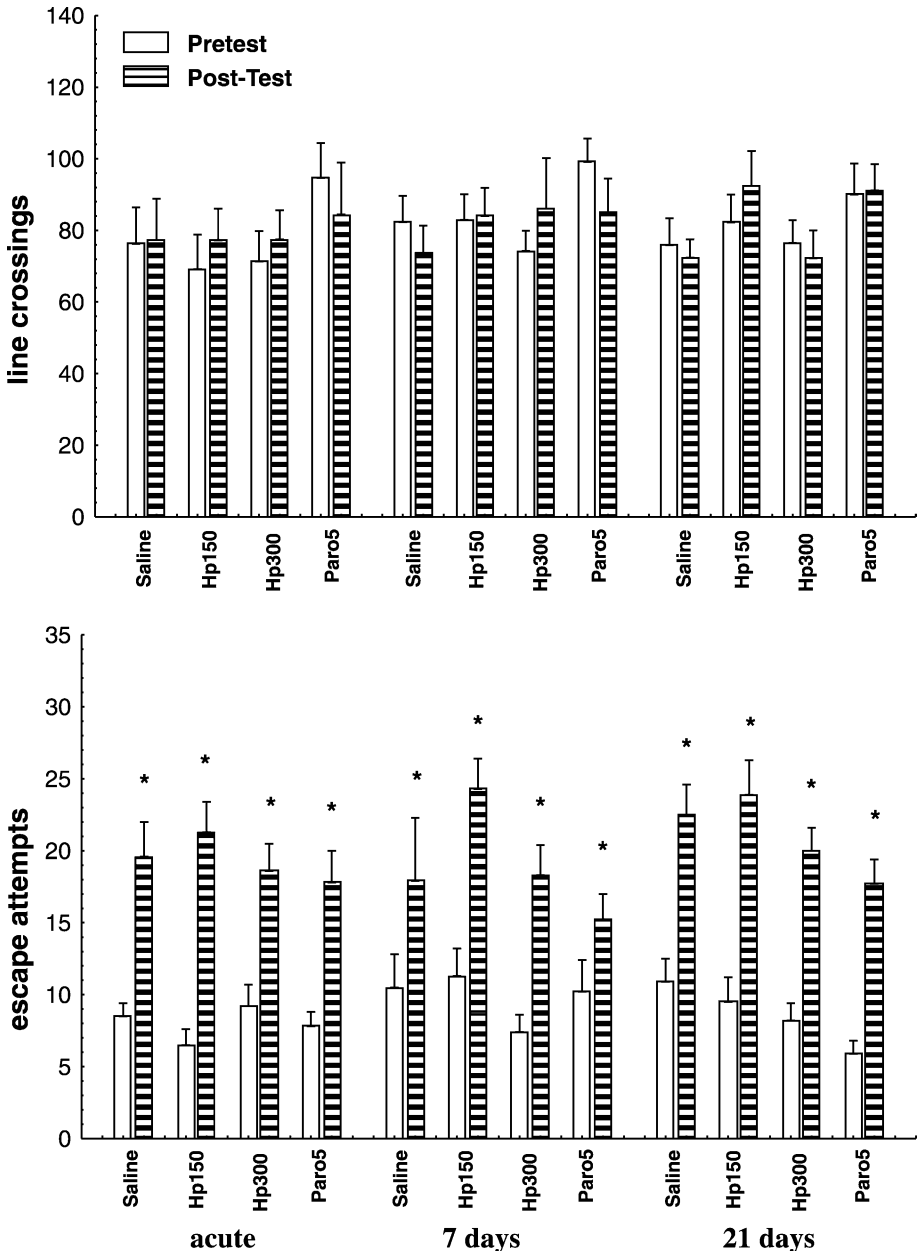

Fig. 1. Effects of acute, subchronic (7 days), and chronic (21 days) treatment with saline (control group), *H. perforatum* 150 mg/kg (Hp 150) and 300 mg/kg (Hp 300) and paroxetine 5 mg/kg (Paro 5) on locomotor activity (line crossings) and escape attempts in the runway cage before (pretest) and after (post-test) confrontation with the rat. Data are reported as mean ± S.E.M. ($n = 14–17$/group). * $P < .05$ compared to pretest.

2.7. Ethics

All procedures were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals (Committee to Revise the Guide for the Care and Use of Laboratory Animals, 1996).

3. Results

3.1. Pretest and post-test (contextual defense)

For the acute treatment, two-way ANOVA showed that line crossings were not affected by Treatment [$F(3,55) = 0.794$; $P > .1$], by Trial [$F(1,55) = 0.045$; $P > .1$] or by Factor interaction [$F(3,55) = 0.443$; $P > .1$]. After 7 days of administration, there was no significant difference in line crossings between treatments [$F(3,55) = 0.989$; $P > .1$] or trials [$F(1,55) = 0.186$; $P > .1$] or in terms of Factor interaction [$F(3,55) = 0.937$; $P > .1$]. The same results were obtained after chronic Treatment [$F(3,55) = 2.271$; $P = .088$], Trial [$F(1,55) = 0.026$; $P > .1$], and Factor interaction [$F(3,55) = 0.415$; $P > .1$] (Fig. 1).

Acute treatment did not affect escape attempts before [$H(3,59) = 1.793$; $P > .1$] or after confrontation with the rat [$H(3,59) = 1.931$; $P > .1$]. The Wilcoxon matched pair test showed that escape attempts in the contextual defense test increased in all groups compared to the pretest and that the drugs did not inhibit this effect: saline ($T = 3.0$; $P < .01$), Hp 150 ($T = 1.0$; $P < .001$), Hp 300 ($T = 9.5$; $P < .01$), and Paro 5 ($T = 5.0$; $P < .01$). Subchronic treatment did not affect escape attempts in the pretest [$H(3,59) = 4.008$; $P > .1$], but affected

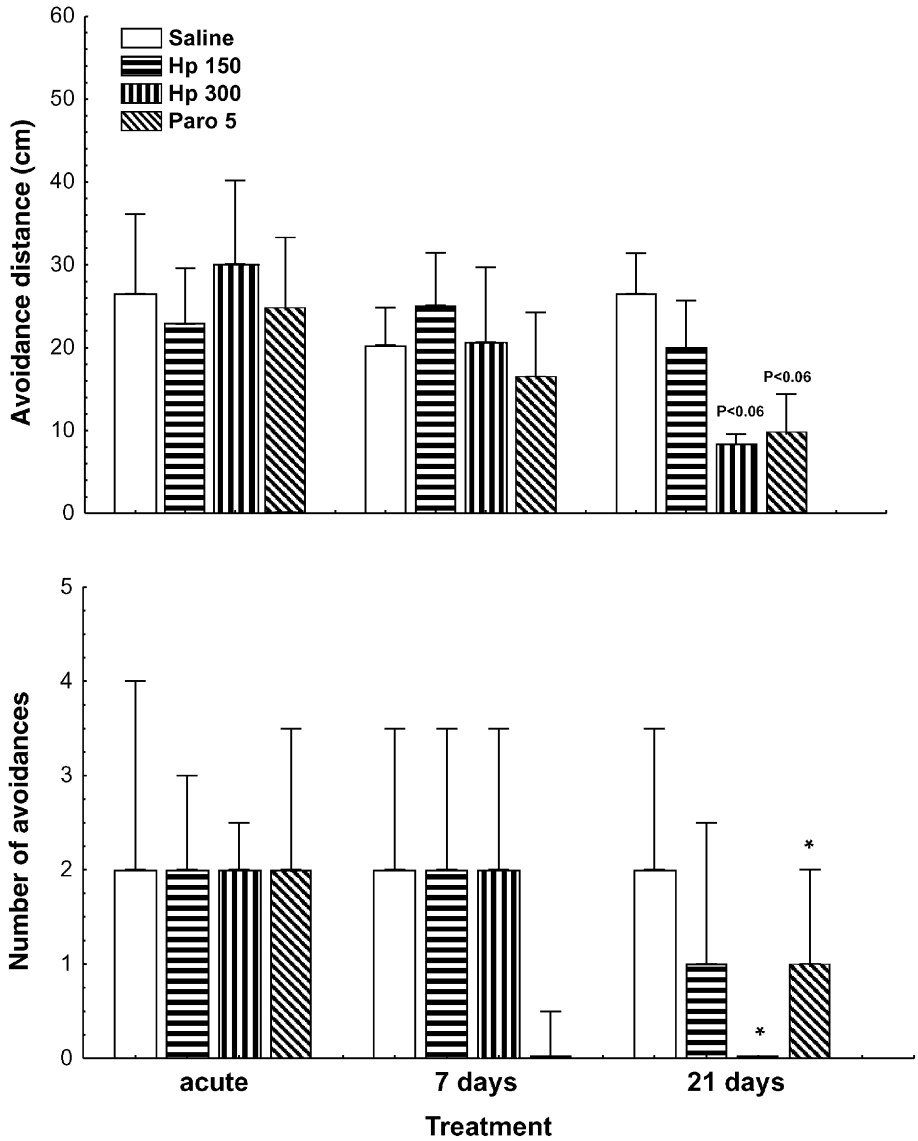

Fig. 2. Effects of acute, subchronic (7 days), and chronic (21 days) treatment with saline (control group), *H. perforatum* 150 mg/kg (Hp 150) and 300 mg/kg (Hp 300) and paroxetine 5 mg/kg (Paro 5) on two flight measures in the rat avoidance test. Number of avoidances is reported as mean ± S.E.M.; avoidance distance data are reported as median ± S.I.R. ($n = 14–17$/group). * $P < .05$ compared to the control group.

this parameter in the post-test [$H(3,59)=9.565$; $P=.022$]. Post hoc Dunn's test indicated a significant difference between Hp 150 and Paro 5. The Wilcoxon matched-pair test showed that escape attempts in the contextual defense test increased in all groups compared to the pretest and that the drugs did not inhibit this effect: saline ($T=22.0$; $P=.03$), Hp 150 ($T=3.5$; $P<.01$), Hp 300 ($T=1.0$; $P<.01$), and Paro 5 ($T=19.5$; $P=.02$). Kruskal–Wallis ANOVA showed that chronic treatment did not affect the number of escape attempts before [$H(3,66)=5.616$; $P>.1$] or after presentation of the predator [$H(3,66)=6.578$; $P=.086$]. The Wilcoxon matched-pair test showed that escape attempts in the contextual defense test increased in all groups compared to the pretest and that the drugs did not inhibit this effect: saline ($T=0.0$; $P<.01$), Hp 150 ($T=1.0$; $P<.001$), Hp 300 ($T=1.0$; $P<.01$), and Paro 5 ($T=0.0$; $P<.01$).

3.2. The rat avoidance test

Statistical analysis showed that acute treatment did not affect the number of avoidances [$H(3,59)=1.123$; $P>.1$] or the avoidance distance [$F(3,55)=0.123$; $P>.1$]. Subchronic treatment also did not affect the number of avoidances [$H(3,59)=7.016$; $P=.071$] or the avoidance distance [$F(3,55)=0.250$; $P>.1$]. After chronic administration, there was a significant difference between groups in number of avoidances [$H(3,66)=15.210$; $P=.001$] and avoidance distance [$F(3,62)=3.026$; $P=.036$]. Post hoc analysis (Dunn's test) showed that Hp 300 ($P<.01$) and Paro 5 ($P<.01$) significantly reduced the number of avoidances compared to the control group. Moreover, Hp 300 and Paro 5 also reduced the avoidance distance, but this reduction just failed to reach statistical significance ($P<.06$) (Fig. 2).

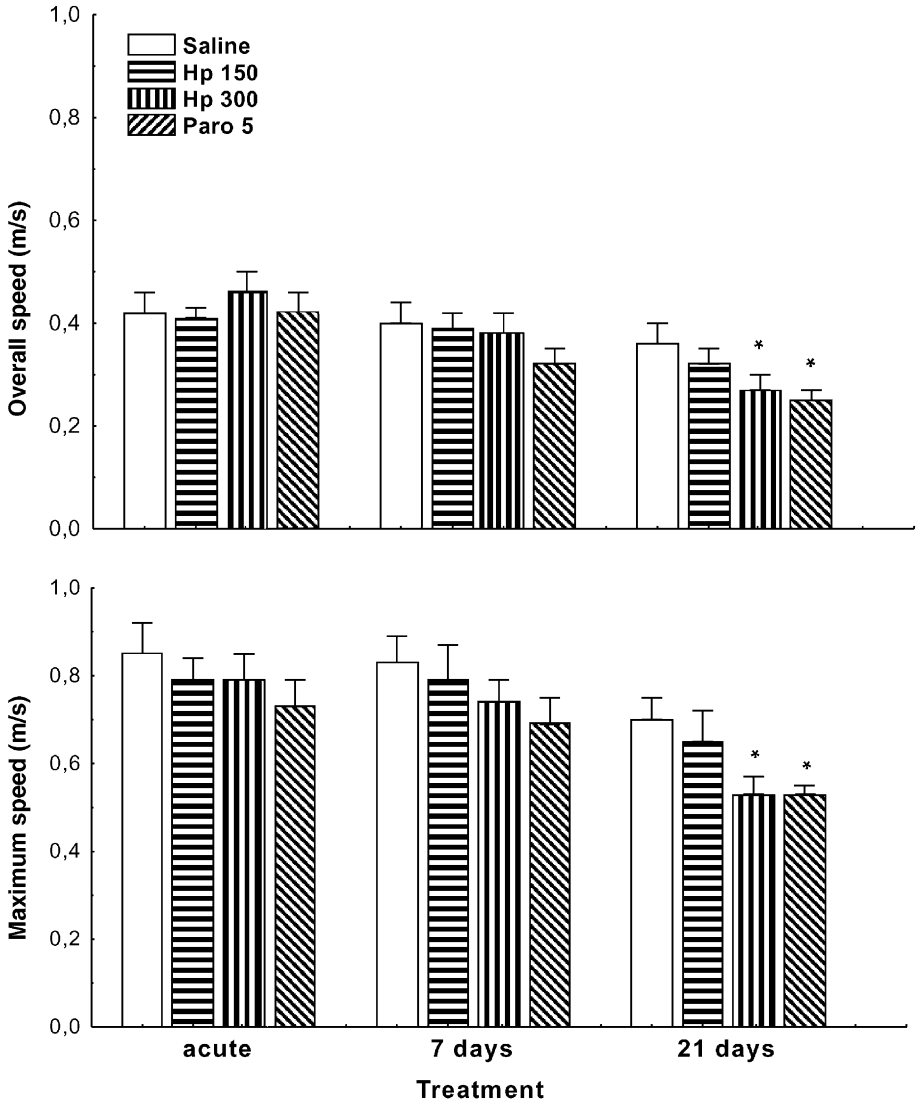

Fig. 3. Effects of acute, subchronic (7 days), and chronic (21 days) treatment with saline (control group), *H. perforatum* 150 mg/kg (Hp 150) and 300 mg/kg (Hp 300) and paroxetine 5 mg/kg (Paro 5) on overall and maximum speed in the chase/flight test. Data represent the mean ± S.E.M. ($n=14–17$/group). * $P<.05$ compared to the control group.

3.3. The chase/flight test

Overall [$F(3,55) = 0.018$; $P > .1$] and maximum [$F(3, 55) = 0.054$; $P > .1$] speed (Fig. 3), number of stops [$H(3, 59) = 1.089$; $P > .1$], number of orientations [$H(3,59) = 0.075$; $P > .1$] and number of reversals [$H(3,59) = 1.111$; $P > .1$; Table 1] were not affected by acute treatment. After subchronic treatment, overall [$F(3,55) = 1.319$; $P > .1$] and maximum [$F(3,55) = 0.063$; $P > .1$] speed, number of stops [$H(3,59) = 0.215$; $P > .1$], number of orientations [$H(3,59) = 1.859$; $P > .1$], and number of reversals [$H(3,59) = 4.130$; $P > .1$] remained unchanged in all groups. After chronic treatment, with the exception of Hp 150, the drugs significantly decreased overall [$F(3,62) = 3.363$; $P = .024$] and maximum [$F(3,62) = 3.981$; $P = .011$] speed (Fig. 3). In contrast, the number of stops [$H(3,59) = 1.958$; $P > .01$], the number of orientations [$H(3,59) = 2.798$; $P > .1$], and the number of reversals [$H(3,59) = 1.895$; $P > .1$] were not affected by chronic treatment (Table 1).

3.4. The straight alley test

Immobility time [$F(3,55) = 0.029$; $P > .1$], closest distance between the mouse and the rat [$H(3,59) = 0.950$; $P > .1$], and number of approaches/withdrawals [$H(3,59) = 0.301$; $P > .1$] were not affected by acute treatment. After subchronic treatment, closest distance between the mouse and the rat [$H(3,59) = 7.535$; $P = .056$] and number of approaches/withdrawals [$H(3,59) = 5.592$; $P > .1$] remained unchanged in all groups, but there was a significant difference between groups in immobility time [$F(3,55) = 3.214$; $P = .029$]. Post hoc analysis by the Newman–Keuls test showed a significant difference between Hp 300 and Paro 5. After chronic treatment, immobility time [$F(3,62) = 1.417$; $P > .1$] and closest distance between the mouse and the rat [$H(3,66) = 7.229$; $P = .065$] remained unchanged in all groups, but there was a significant difference between groups in number of approaches/withdrawals [$H(3,66) = 9.380$; $P = .024$]. The Dunn test showed that Paro 5 administration significantly increased the number of approaches/withdrawals compared to the control group ($P < .05$) (Table 2).

Table 1
Effects of *H. perforatum* and paroxetine on the frequencies of behaviors in the chase/fight test

Treatment	Stops	Orientations	Reversals
Acute			
Saline ($n = 15$)	11.0 ± 4.5	5.0 ± 5.5	3.0 ± 7.5
Hp 150 ($n = 15$)	12.0 ± 4.0	5.0 ± 5.0	5.0 ± 5.0
Hp 300 ($n = 15$)	11.0 ± 2.0	6.0 ± 2.5	4.0 ± 3.5
Paro 5 ($n = 14$)	10.5 ± 2.5	5.0 ± 1.0	4.5 ± 4.0
7 days			
Saline ($n = 15$)	15.0 ± 2.5	10.0 ± 4.5	4.0 ± 4.5
Hp 150 ($n = 15$)	15.0 ± 3.0	8 ± 3.5	1.0 ± 4.5
Hp 300 ($n = 14$)	14.5 ± 2.5	8.0 ± 2.0	3.0 ± 1.5
Paro 5 ($n = 15$)	14.0 ± 4.5	10.0 ± 1.5	2.0 ± 3.0
21 days			
Saline ($n = 16$)	12.5 ± 2.0	9.0 ± 2.25	1.0 ± 0.5
Hp 150 ($n = 17$)	15.0 ± 1.5	11.0 ± 2.0	1.0 ± 0.5
Hp 300 ($n = 17$)	15.0 ± 3.0	12.0 ± 3.5	1.0 ± 0.5
Paro 5 ($n = 16$)	15.5 ± 4.0	11.0 ± 4.0	2.0 ± 1.0

Data represent the median ± S.I.R.
Hp 150 and Hp 300: *H. perforatum* 150 and 300 mg/kg, respectively; Paro 5: paroxetine 5 mg/kg. Drugs were administered orally.

Table 2
Effects of *H. perforatum* and paroxetine in the straight alley test on behavioral responses to a rat

Treatment	Immobility time[a]	Closest distance between animals[b]	Approaches/ withdrawals[b]
Acute			
Saline ($n = 15$)	27.3 ± 3.2	40.0 ± 5.0	0.0 ± 0.0
Hp 150 ($n = 15$)	27.6 ± 3.5	40.0 ± 10.0	0.0 ± 0.0
Hp 300 ($n = 15$)	28.2 ± 3.1	40.0 ± 20.0	0.0 ± 0.0
Paro 5 ($n = 14$)	26.9 ± 2.3	40.0 ± 5.0	0.0 ± 0.0
7 days			
Saline ($n = 15$)	26.7 ± 3.0	40.0 ± 20.0	0.0 ± 0.5
Hp 150 ($n = 15$)	19.8 ± 3.8	40.0 ± 20.0	0.0 ± 0.5
Hp 300 ($n = 14$)	29.8 ± 1.8	40.0 ± 0.0	0.0 ± 0.0
Paro 5 ($n = 15$)	17.3 ± 3.7	20.0 ± 20.0	0.0 ± 0.5
21 days			
Saline ($n = 16$)	24.6 ± 2.7	40.0 ± 15.0	0.0 ± 0.0
Hp 150 ($n = 17$)	19.4 ± 3.7	25.0 ± 20.0	0.0 ± 0.0
Hp 300 ($n = 17$)	20.6 ± 3.2	40.0 ± 15.0	0.0 ± 0.0
Paro 5 ($n = 16$)	15.0 ± 3.3	0.0 ± 13.75	1.0 ± 2.0 *

Hp 150 and Hp 300: *H. perforatum* 150 and 300 mg/kg, respectively; Paro 5: paroxetine 5 mg/kg. Drugs were administered orally.
[a] Data represent the mean ± S.E.M.
[b] Data represent the median ± S.I.R.
* $P < .05$ compared to the control group.

Table 3
Effects of *H. perforatum* and paroxetine on frequencies of defensive threat and attack reactions upon forced contact with a rat

Treatment	Biting the rat	Vocalizations	Jump attacks	Upright postures
Acute				
Saline ($n = 15$)	0.0 ± 1.0	4.0 ± 5.0	1.0 ± 4.0	8.0 ± 2.5
Hp 150 ($n = 15$)	0.0 ± 0.5	2.0 ± 3.5	0.0 ± 1.0	9.0 ± 4.5
Hp 300 ($n = 15$)	0.0 ± 0.5	0.0 ± 3.5	0.0 ± 5.5	8.0 ± 4.5
Paro 5 ($n = 14$)	0.0 ± 0.5	0.5 ± 2.5	0.5 ± 2.5	5.0 ± 4.5
7 days				
Saline ($n = 15$)	0.0 ± 0.5	11.0 ± 6.5	2.0 ± 2.0	14.0 ± 3.5
Hp 150 ($n = 15$)	0.0 ± 0.0	5.0 ± 17.0	0.0 ± 3.5	12.0 ± 8.5
Hp 300 ($n = 14$)	0.0 ± 0.0	11.0 ± 9.5	0.0 ± 1.5	9.0 ± 4.0
Paro 5 ($n = 15$)	0.0 ± 0.0	2.0 ± 9.5	0.0 ± 0.5	7.0 ± 6.5
21 days				
Saline ($n = 16$)	0.0 ± 0.0	5.0 ± 9.5	0.0 ± 0.5	24.0 ± 5.25
Hp 150 ($n = 17$)	0.0 ± 0.0	7.0 ± 15.0	0.0 ± 0.0	8.0 ± 11.0
Hp 300 ($n = 17$)	0.0 ± 0.0	1.0 ± 7.5	0.0 ± 0.0	10.0 ± 4.5 *
Paro 5 ($n = 16$)	0.0 ± 0.0	0.5 ± 4.25	0.0 ± 0.0	7.0 ± 6.0 *

Data represent the median ± S.I.R.
Hp 150 and Hp 300: *H. perforatum* 150 and 300 mg/kg, respectively; Paro 5: paroxetine 5 mg/kg. Drugs were administered orally.
* $P < .05$ compared to the control group.

3.5. The forced contact test

Number of bites [$H(3,59) = 2.292$; $P > .1$], vocalizations [$H(3,59) = 5.354$; $P > .1$], jump attacks [$H(3,59) = 1.127$; $P > .1$] and upright postures [$H(3,59) = 1.671$; $P > .1$] were not affected by acute treatment. After subchronic treatment, number of bites [$H(3,59) = 7.466$; $P = .058$], vocalizations [$H(3,59) = 0.490$; $P > .1$], jump attacks [$H(3,59) = 3.036$; $P > .1$], and upright postures [$H(3,59) = 1.751$; $P > .1$] also remained unchanged in all groups. The number of bites [$H(3,66) = 2,039$; $P > .1$] and vocalizations [$H(3,66) = 3.300$; $P > .1$] were not affected by chronic treatment, but the number of jump attacks [$H(3,66) = 7.956$; $P = .046$] and upright postures [$H(3,66) = 13.334$; $P = .004$] were. Post hoc analysis by Dunn's test showed that Hp 300 and Paro 5 significantly reduced the frequency of upright postures compared to the control group. On the other hand, Dunn's test did not reveal any significant difference between groups for jump attacks (Table 3).

4. Discussion

The defensive behaviors of control animals in the present study were qualitatively similar to those reported in previous MDTB studies (Griebel et al., 1995a, 1996b). For example, the escape attempts were increased by previous confrontation with the predator. On the other hand, the baseline levels of defensive behaviors in the present study were lower than previously reported in the literature. A possible explanation for these differences may be the use of a less aggressive strain of rats as a threatening stimulus (Wistar in the present study versus Long Evans in previous studies). Another possibility is that our mice were housed in groups and not singly as in previous MDTB studies; it is acknowledged that isolation promotes defensive and offensive aggression. Nevertheless, in the present study, defensive behaviors also were elicited despite their lower intensity compared to previous studies and despite the intertrial variation found here.

Chronic treatment with *H. perforatum* 300 mg/kg and paroxetine 5 mg/kg significantly reduced flight responses (number of avoidances and overall flight speed). Considering that these behaviors are highly sensitive to panicogenic and panicolytic agents (Blanchard et al., 1997, 2001), the effects of paroxetine, a clinically effective antipanic drug, substantiate the proposal that MDTB is a useful animal model for the screening of antipanic-like drugs. Along this line, clinical studies have shown an antipanic effect of paroxetine only after repeated administration (Lecrubier et al., 1997; Lecrubier and Judge, 1997; Ballenger et al., 1998). Thus, the results obtained with *H. perforatum* suggest an antipanic-like effect of this drug. This agrees with the data showing that both drugs increased one-way escape latency of rats subjected to the elevated T-maze with a modified procedure after repeated administration (Beijamini and Andreatini, unpublished data), a finding that could be interpreted as an antipanic-like effect (Graeff et al., 1998). However, as described above, another study did not find impairment in one-way escape in the elevated T-maze (Flausino et al., 2002). There is one clinical case report showing that *H. perforatum* treatment reduced the number of panic attacks in a patient with possible PD (Yager et al., 1999), which may weakly suggest a potential antipanic effect of *H. perforatum*.

H. perforatum or paroxetine treatment did not affect the number of escape attempts before and after presentation of the predator. Moreover, the results showed that escape attempts increased in all groups and that the drugs were not able to inhibit this effect. The paroxetine results are in contrast to the data reported by Griebel et al. (1995a), which showed that chronic fluoxetine or imipramine treatment significantly reduced the frequency of escape attempts compared to the control group, an effect interpreted as a partial anxiolytic-like effect. Furthermore, neither *H. perforatum* nor paroxetine affected risk assessment behaviors in the chase/flight test after the different treatments, although chronic imipramine and fluoxetine reduced the number of stops and orientations (Griebel et al., 1995a). Because these behaviors were associated with an anxiolytic-like effect and clinical evidence shows that GAD is improved by repeated treatment with paroxetine (Rocca et al., 1997; Allgulander et al., 1998), the absence of an effect of repeated paroxetine treatment, at least at the dose used here, on these parameters of the MDTB could be viewed as a false-negative effect. Nevertheless, paroxetine significantly increased the number of approaches/withdrawals. Recently, Griebel et al. (1999a,b) observed that diazepam increases the frequency of this behavior in Swiss mice and they suggested that this may be an anxiolytic-like effect. Moreover, both paroxetine and *H. perforatum* extract reduced the number of upright postures. Vocalizations, bites, upright postures, and jump attacks are defensive threat and attack reactions that have been found to be sensitive to anxiolytic drugs and that are consequently related to GAD (Blanchard et al., 1997, 1998). Thus, we could interpret these results as both paroxetine and the extract exerting a partial anxiolytic-like effect in the MDTB. Another difference between our results and previous data is the absence of an anxiogenic-like effect of acute treatment with paroxetine, in contrast to the anxiogenic-like effect found with acute treatment with fluoxetine and imipramine. Although in PD, an anxiogenic effect sometimes is seen at the beginning of antidepressant treatment (e.g., with fluoxetine and clomipramine), this effect was not seen in clinical studies with paroxetine (e.g., Lecrubier et al., 1997; Ballenger et al., 1998). Moreover, as cited above, the methodological differences between studies (housing conditions and rat strain) also may have contributed to these discrepancies. Furthermore, it is important to note that paroxetine was tested with only one dose, a fact that makes it difficult to draw consistent conclusions. Thus, we think that these differences between the paroxetine

results and previous data do not reduce the validity and utility of the MDTB.

An alternative hypothesis explaining the anxiolytic/antipanic-like effect is that the drug treatment modified locomotor activity. This hypothesis, however, can be partially discounted, because acute, subchronic, and chronic treatments with *H. perforatum* or paroxetine did not affect the line crossing frequencies before confrontation with the rat. In previous studies with MDTB, line crossing frequency during the pretest condition was used as a measure of spontaneous locomotor activity (Griebel et al., 1995b, 1999a; Blanchard et al., 1999). The results suggest that these drugs did not change locomotor activity. This conclusion is strengthened by the results of our previous experiment which showed that acute treatment with *H. perforatum* did not modify locomotor activity (beam interruptions) of mice tested in automated chambers (Skalisz et al., unpublished data).

Other animal studies also strengthen the hypothesis that *H. perforatum* elicits an anxiolytic-like effect in the MDTB. As described in the Introduction, the *H. perforatum* extract exhibits an anxiolytic-like effect on several animal models of anxiety: the light–dark test in rats (Vandenbogaerde et al., 2000; Flausino et al., 2002), the elevated T-maze (Flausino et al., 2002; Beijamini and Andreatini, unpublished data), the marble-burying behavior in mice (Skalisz et al., unpublished data), the elevated plus-maze (Kumar et al., 2000; Coleta et al., 2001), and social interaction (Kumar et al., 2000).

The *H. perforatum* extract has several biologically active constituents possibly acting on many neurotransmitter systems that could explain its effects. On this basis, it was found that hyperforin induces an anxiolytic-like effect on the elevated plus-maze, an animal model of anxiety (Chatterjee et al., 1998b). However, another study has shown that an *H. perforatum* extract without hyperforin increases the time spent in the open arms of the elevated plus-maze (Coleta et al., 2001), suggesting an anxiolytic-like effect of other *H. perforatum* constituents. There are some data suggesting that, like paroxetine, fluoxetine, and imipramine, *H. perforatum* extract (or hyperforin) could inhibit serotonin re-uptake (Muller et al., 1998; Di Carlo et al., 2001; Nathan, 2001), which could explain the effects found in the MDTB. However, recent data do not corroborate this serotonergic effect (Gobbi et al., 1999; Fornal et al., 2001). On the other hand, the anxiolytic-like effect of *H. perforatum* in the light–dark test was blocked by pretreatment with flumazenil, a benzodiazepine receptor antagonist (Vandenbogaerde et al., 2000), a fact suggesting possible GABAergic modulation. Thus, additional studies are needed to determine the anxiolytic/antipanic-like effects of *H. perforatum*.

In conclusion, the present results showed that chronic *H. perforatum* extract reduced some flight behaviors in the MDTB, which has been suggested to be primarily sensitive to antipanic agents. However, these effects were smaller than those observed in previous studies with clinically effective antipanic drugs. Moreover, there is a conflicting result when the present data are compared to those obtained by Flausino et al. (2002) with the elevated T maze, another animal model proposed to evaluate panic-related anxiety. Thus, additional studies are needed in order to confirm the potential antipanic-like effect of *H. perforatum* extract.

Acknowledgements

RA is the recipient of a Brazilian National Research Council (CNPq) research fellowship and VB was the recipient of a CAPES postgraduate fellowship. We thank Dr. Guy Griebel for help with the standardization of the MDTB procedure in our laboratory, Dr. Hidevaldo B. Machado for his comments on the manuscript, Mrs. Silvia Cordazzo Genaro for technical assistance, and Eurofarma (Brazil) for the supply of *H. perforatum* extract and paroxetine. This study was partially supported by FUNPAR.

References

Allgulander C, Cloninger CR, Przybeck TR, Brandt L. Changes on the temperament and character inventory after paroxetine treatment in volunteers with generalized anxiety disorder. Psychopharmacol Bull 1998; 34:165–6.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington (DC): American Psychiatric Association; 1994.

Ballenger JC, Wheadon DE, Steiner M, Bushnell W, Gergel IP. Double blind, fixed-dose, placebo-controlled study of paroxetine in the treatment of panic disorder. Am J Psychiatry 1998;155:36–42.

Bhattacharya SK, Chakrabarti A, Chatterjee SS. Activity profiles of two hyperforin-containing *Hypericum* extracts in behavioral models. Pharmacopsychiatry 1998;31(Suppl 1):22–9.

Blanchard RJ, Yudko EB, Rodgers RJ, Blanchard DC. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behav Brain Res 1993a;58:155–65.

Blanchard RJ, Taukulis HK, Rodgers RJ, Magee LK, Blanchard DC. Yohimbine potentiates active defensive responses to threatening stimuli in Swiss–Webster mice. Pharmacol Biochem Behav 1993b;44:673–81.

Blanchard RJ, Griebel G, Henrie JA, Blanchard DC. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci Biobehav Rev 1997;21:783–9.

Blanchard DC, Griebel G, Rodgers RJ, Blanchard RJ. Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. Neurosci Biobehav Rev 1998;22:597–612.

Blanchard RJ, Kaawaloa JN, Herbert MA, Blanchard DC. Cocaine produces panic-like flight responses in mice in the mouse defense test battery. Pharmacol Biochem Behav 1999;64:523–8.

Blanchard DC, Griebel G, Blanchard RJ. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci Biobehav Rev 2001;25:205–18.

Butterweck V, Wall A, Liefländer-Wulf U, Winterhoff H, Nahstedt A. Effects of total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. Pharmacopsychiatry 1997;30 (Suppl 2):117–24.

Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A, Müller WE. Hyperforin as a possible antidepressant component of *Hypericum* extracts. Life Sci 1998a;63:499–510.

Chatterjee SS, Noldner M, Koch E, Erdelmeier C. Antidepressant activity

of *Hypericum perforatum* and Hyperforin: the neglected possibility. Pharmacopsychiatry 1998b;31(Suppl 1):7–15.

Coleta M, Campos MG, Cotrim MD, Proença da Cunha A. Comparative evaluation of *Melissa officinalis* L., *Tilia europaea* L., *Passiflora edulis* Sims. and *Hypericum perforatum* L. in the elevated plus maze anxiety test. Pharmacopsychiatry 2001;34(Suppl 1):S20–1.

Committee to Revise the Guide for the Care and Use of Laboratory Animals. 1996 [http://oacu.od.nih.gov/regs/guide/guidex.htm].

Davison JRT, Connor KM. St. John's wort in generalized anxiety disorder: three case reports. J Clin Psychopharmacol 2001;21:635–6.

Deakin JFW, Graeff FG. 5-HT and mechanisms of defense. J Psychopharmacol 1991;5:305–15.

Di Carlo G, Borrelli F, Ernest E, Izzo AA. St. John's wort: Prozac from the plant kingdom. Trends Pharmacol Sci 2001;22:292–7.

Flausino Jr AO, Zangrossi Jr H, Salgado JV, Viana MB. Effects of acute and chronic treatment with *Hypericum perforatum* L. (LI-160) on different anxiety-related responses in rats. Pharmacol Biochem Behav 2002;71:259–65.

Fornal CA, Metzler CW, Mirescu C, Stein SK, Jacobs BL. Effects of standardized extracts of St. John's wort on the single-unit activity of serotonergic dorsal raphe neuron in awake cats: comparisons with fluoxetine and sertraline. Neuropsychopharmacology 2001;25(6):858–70.

Gambarana C, Ghiglieri O, Tolu P, De Montis MG, Giachetti D, Bombardelli E, et al. Efficacy of an *Hypericum perforatum* (St. John's wort) extract in preventing and reverting a condition of escape deficit in rats. Neuropsychopharmacology 1999;21:247–57.

Gobbi M, Valle FD, Ciapparelli C, Diomede L, Morazzoni P, Verotta L, et al. *Hypericum perforatum* L. extract does not inhibit 5-HT transporter in rat brain cortex. Naunyn-Schmiedeberg's Arch Pharmacol 1999;360:262–9.

Graeff FG, Netto CF, Zangrossi Jr H. The elevated T-maze as an experimental model of anxiety. Neurosci Biobehav Rev 1998;23:237–46.

Griebel G, Blanchard DC, Agnes RS, Blanchard RJ. Differential modulation of antipredator defensive behavior in Swiss–Webster mice following acute and chronic administration of imipramine and fluoxetine. Psychopharmacology 1995a;120:57–66.

Griebel G, Blanchard DC, Jung A, Blanchard RJ. A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. Behav Pharmacol 1995b;6:732–45.

Griebel G, Blanchard DC, Jung A, Masuda CK, Blanchard RJ. 5-HT_{1A} agonists modulate mouse antipredator defensive behavior differently from the 5-HT_{2A} antagonist pirenperone. Pharmacol Biochem Behav 1995c;51:235–44.

Griebel G, Blanchard DC, Jung A, Lee JC, Masuda CK, Blanchard RJ. Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 1995d;34:1625–33.

Griebel G, Blanchard DC, Blanchard RJ. Predator-elicited flight responses in Swiss–Webster mice: an experimental model of panic attacks. Prog Neuro-Psychopharmacol Biol Psychiatry 1996a;20:185–205.

Griebel G, Sanger DJ, Perrault G. The mouse defense test battery: evaluation of the effects of non-selective and BZ-1 (ϖ1) selective, benzodiazepine receptor ligands. Behav Pharmacol 1996b;6:560–72.

Griebel G, Blanchard DC, Blanchard RJ. Evidence that the behaviors in the mouse defense test battery relate to different emotional states: a factor analytic study. Physiol Behav 1996c;60:1255–60.

Griebel G, Perrault G, Sanger DJ. Behavioural profiles of the reversible monoamino-oxidase-A inhibitors beflloxatone and moclobemide in an experimental model for screening anxiolytic and antipanic drugs. Psychopharmacology 1997a;131:180–6.

Griebel G, Perrault G, Sanger DJ. A comparative study of the effects of selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 1997b;36:793–802.

Griebel G, Curet O, Perrault G, Sanger DJ. Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: correlation with changes in monoamine-oxidase activity and monoamine levels. Neuropharmacology 1998;37:927–35.

Griebel G, Rodgers RJ, Perrault G, Sanger DJ. Behavioural profiles in the mouse defense test battery suggest anxiolytic potential of 5-HT_{1A} receptor antagonists. Psychopharmacology 1999a;144:121–30.

Griebel G, Perrault G, Sanger DJ. Differences in anxiolytic-like profile of two novel nonbenzodiazepine BZ (ω) receptor agonists on defensive behaviors of mice. Pharmacol Biochem Behav 1999b;62:689–94.

Kasper S. *Hypericum perforatum*—a review of clinical studies. Pharmacopsychiatry 2001;34(Suppl 1):S51–5.

Kim HL, Streltzer J, Goebert D. St. John's wort for depression: a meta-analysis of well-defined clinical trials. J Nerv Ment Dis 1999;187:532–8.

Kumar V, Jaiswal AK, Singh PN, Bhattacharya SK. Anxiolytic activity of Indian *Hypericum perforatum* Linn: an experimental study. Indian J Exp Biol 2000;38:36–41.

Lecrubier Y, Judge R. Long-term evaluation of paroxetine, clomipramine and placebo in panic disorder. Acta Psychiatr Scand 1997;95:153–60.

Lecrubier Y, Bakker A, Dunbar G, Judge R. A comparison of paroxetine, clomipramine and placebo in the treatment of panic disorder. Acta Psychiatr Scand 1997;95:145–52.

Linde K, Mulrow CD. St. John's wort for depression. Cochrane Database Syst Rev 2000:2:CD000448.

Muller WE, Rolli M, Schafer C, Hafner U. The effects of *Hypericum* extract (LI 160) in biochemical models of antidepressant activity. Pharmacopsychiatry 1997;30(Suppl 2):102–7.

Muller WE, Singer A, Wonnemann M, Hafner U, Rolli M, Schafer C. Hyperforin represents the neurotransmitter reuptake inhibiting constituent of *Hypericum* extract. Pharmacopsychiatry 1998;3(Suppl 1):16–21.

Nathan PJ. *Hypericum perforatum* (St. John's Wort): a non-selective reuptake inhibitor? A review of the recent advances in its pharmacology. J Psychopharmacol 2001;15(1):47–54.

Rocca P, Fonzo V, Scotta M, Zanalda E, Ravizza L. Paroxetine efficacy in the treatment of generalized anxiety disorder. Acta Psychiatr Scand 1997;95:444–50.

Shelton RC, Keller MB, Gelemberg A, Dunner DL, Hirschfeld R, Thase ME, et al. Effectiveness of St. John's wort in major depression: a randomized controlled trial. JAMA 2001;285:1978–86.

Stevinson C, Dixon M, Ernst E. *Hypericum* for fatigue—a pilot study. Phytomedicine 1998;5:443–7.

Taylor LH, Kobak KA. An open-label trial of St. John's wort (*Hypericum perforatum*) in obsessive–compulsive disorder. J Clin Psychiatry 2000;61:575–8.

Vandenbogaerde A, Zanoli P, Puia G, Truzzi C, Kamuhabwa A, De Witte P, et al. Evidence that total extract of *Hypericum perforatum* affects exploratory behavior and exerts anxiolytic effects in rats. Pharmacol Biochem Behav 2000;65:627–33.

Wonnemann M, Singer A, Siebert B, Muller WE. Evaluation of synaptosomal uptake inhibition of most relevant constituents of St. John's wort. Pharmacopsychiatry 2001;34(Suppl 1):S148–51.

Yager J, Siegfreid MD, Di Matteo TL. Use of alternative remedies by psychiatric patients: illustrative vignettes and a discussion of the issues. Am J Psychiatry 1999;156:1432–8.