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Antidepressant activity evaluation of *Hypericum brasiliense* standardized extract

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ABSTRACT

Hypericum brasiliense (Hypericaceae) is a Brazilian traditional plant used as an excitant, antispasmodic and antiofidic agent in the South and Southeastern areas. Pharmacological studies were performed to evaluate antidepressant effects of standard extract of *H. brasiliense* (SEHB) alone or combined with fluoxetine (10 mg/kg *i.p.*), GBR-12909 (10 mg/kg *ip*) or Trans-2-phenylcyclopropylamine (5 mg/kg *ip*) using forced swimming test (FST), open field test (OFT) and rota rod assays. In the FST, SEHB reduced in a dose-dependent manner the immobility time, and has shown antagonistic effect when administrated with fluoxetine. In the OFT, SEHB has caused marginal effect of the evaluated parameters (ambulation and rearing), but when associated with fluoxetine or trans-2-phenylcyclopropylamine, the reduction of the parameters was noticed. On rota rod test, SEHB did not produce significant alteration. Based on results we suggest that SEHB has an antidepressant activity.

KEYWORDS: *Hypericum brasiliense*; Hypericaceae, Forced swimming test (FST); Open field test (OFT); Antidepressant.

INTRODUCTION

Hypericum perforatum is the most commonly and known specie from Hypericaceae family. The *Hypericum* genus has been popularly used to treatment of depression (1; 2; 3; 4), pain (5), inflammatory injuries (6; 7) and others diseases. The *H. brasiliense* is a widely Brazilian South and Southeastern plant used by population as an excitant, antispasmodic and against snake bite (8). Moreover, xanthones and flavonoids isolated from the dichloromethane and methanol extracts of *H. brasiliense* respectively, have shown *in vitro* IMAO activity, with MAO_A selectively (9, 10). Some authors have described negative results for antidepressant activity with intraperitoneal treatment in the forced swimming test (11). Recently, the hydroalcoholic extract of *H. brasiliense* has shown no antidepressant activity using the forced swimming test and hypothermia induced by apomorphine. However, the *H. brasiliense* extract used was not standardized for xanthones and flavonoids contents (12). In the present work, the standardized extract of *Hypericum brasiliense* used is mainly composed by xanthones and flavonoids (9, 10, 13). The aim of this study was to evaluate the antidepressant activity and to delineate the mechanism of action of the standardized extract of *Hypericum brasiliense* using forced swimming and open filed tests.

MATERIAL AND METHODS

Plant material

The plant was collected in the cities of Nova Friburgo and Itatiaia, RJ, Brazil. A voucher specimen was identified by a botanical specialist, Ana Angélica Monteiro de Barros and was deposited at the Botany Department of Biology Institute of Federal University of Rio de Janeiro (number RFA 23.257). The aerial parts of plants were dried at room temperature protected of direct light and pulverized.

Extraction procedure and standardization of extract

The dried material was extracted successively at room temperature with hexane followed by ethanol to furnish an enriched phenolic compound extract (xanthones and flavonoids). The ethanolic extract was dried in a rotaevaporator under reduced pressure. Standardization was proceed using TLC plates 60 GF_{254} (Merck Co.) and HPLC analysis. TLC analysis was made using Merck Co. 60 GF_{254} TLC plates eluted with a

mixture of ethyl acetate: formic acid: acetic acid: chloroform (30:10:10:15) and reveled with NP/PEG and UV_{365}. HPLC analysis was carried out on Shimadzu HPLC system (LC-10AD pumps, SPD-M10A DAD UV/Vis detector). It was performed using binary gradient system composed by water: acetonitrile with 0.05 % of TFA (9:1 to 4:6 in 30 min. – 1 ml/min.), C_{18} column (250 x 4.6 mm – 5 μm, RP-18 Hypersil). Standards flavonoids (kaempferol, hyperine, rutine, isoquercetrine, quercetin, hyperoside and guaijaverine and chlorogenic acid) were used for qualitative analisys. It was performed using previous hydrolysated acid extract and quercetin as standard flavonoid using HPLC method. The calibration curve was obtained using 20 μL of quercetin standard solution, injected at 1.00, 0.75, 0.50 and 0.25 mg/mL.

Chemicals and treatments

Fluoxetine (10 mg/kg, i.p., Sigma Co.), GBR-12909 (10 mg/kg, i.p., Sigma Co.), tranylcypromine (5 mg/kg, i.p. Sigma Co.) were administered as positive controls. These chemicals were solubilized in saline solution at 0.9 % with 2.0 % of polysorbate 80. The volume administered to each animal was 0.5 ml. SEHB was reconstituted with distilled water (0.5 ml/animal) with 2.0 % of polysorbate 80, and administered orally by itself at 150, 300 and 500 mg/kg or with one of the positive control drugs at 500 mg/kg. In all experiments, a negative control group, treated orally with distilled water (0.5 ml), was included.

Animals

Male rats (*Rattus norvegicus*, albinus, Wistar) weighing 180 ± 20 g were used to perform experiments. The animals were kept in polyethylene box (n = 5), in acclimatized environment (23 ± 1^0 C) with circadian cycle of 12 hours and free access to water and food. Animals were taken from the Central Biotery of Alfenas University two weeks before the experimentation to get used with the environment. They were also treated with distilled water during seven days (0.5ml, p.o.) to get used to the treatment and the researcher. All experimental protocols were proceeded with groups of 8 animals and were approved by of Alfenas University Bioethical Committee.

Forced swimming test

Briefly, two successive swimming expositions with a 24 hours brake were preceded using acrylic cylinders (50 cm high x 30 cm diameter) containing water at 25 ºC to a height of 38 cm. During a five minutes swimming period, immobility time and the number of dives were registered. Animals were treated 24, 5 and 1 hour before the test (14).

Open field test

Animals were introduced in an arena (100 cm x 100 cm x 50 cm) with black ground. The ground was divided with white ribbons set in both parallel and perpendicular way with 20 cm spaces among them (15). The experiment was recorded with a video camera and the motion activity (number of squares crossed) and rearing (the animals put hind paws leaning or not on the wall) were counted.

Rota rod test

The test consists to put an animal in a platform with a spinning wheel, divided by circular plates in four compartments (Rota-Rod Treadmill for rats – mod. 7750 – Ugo Basile). Animals were selected 24 hours before the test, and animals not ressisting for 200 seconds in the machine with variable acceleration from 4 to 40 r.p.m. were rejected. The latent time was determined in 30, 60, and 90 minutes after drugs administration (16).

Statistical analysis

Results were expressed as mean ± S.E.M. The statistical significance of any difference in each parameter among the groups was evaluated by one-way ANOVA followed by Dunnett test as *post hoc* test. p values of <0.05, <0.01 and <0.001 were considered statistically significant.

RESULTS

Hypericum brasiliense standardized extract

In the TLC qualitative analysis, the presence of all flavonoids used as standard, except rutin, was observed. The same results were observed in the HPLC qualitative analysis, demonstrating that hyperine, isoquercetrin, quercertin and kaempferol may be used as phytochemical makers in quality control of this species. Quantitative analysis showed that SEHB has an amount by 8.5 % of flavonoids, calculated in quercetin.

Forced swimming test

Treatment with SEHB has shown a dose-dependent decrease of the immobility time. The inhibition was statistical significant to all administered doses ($p < 0.01$). When SEHB was combined with the control drugs, it has increased the effects of GBR-12909 and tranylcypromine, and decreased the effect of fluoxetine. All control drugs were effective in experiment when administered alone. Results are shown in Table 1. Diving response was not modified by any treatment, except by GBR-12909 treatment (results not shown).

Table 1. Effect of Hypericum brasiliense standardized extract (SEHB, p.o.) on forced swimming test and open field test in rats.

Treatment	Dose mg / kg p.o.	Forced Swimming Test	Open Field Test	
		Immobility time (seconds)	Motion activity (number of squares)	Rearing
Control	-----	233.5 ± 12.3	87.5 ± 14.5	22.3 ± 2.1
SEHB	150	198.8 ± 15.9 (**)	52.3 ± 12.0 (**)	20.1 ± 3.6
	300	152.5 ± 8.2 (**)	75.8 ± 9.7	28.0 ± 4.9 (*)
	500	140.3 ± 12.8 (**)	92.4 ± 7.3	39.4 ± 3.4 (**)
FLU	10	157.3 ± 13.5 (**)	31.4 ± 6.1 (**)	11.7 ± 2.1 (**)
FLU + SEHB	10 + 500	226.4 ± 22.0	36.0 ± 10.4 (**)	12.3 ± 3.4 (**)
GBR	10	117.5 ± 11.7 (**)	113.4 ± 9.5 (**)	50.5 ± 3.0 (**)
GBR + SEHB	10 + 500	81.0 ± 7.7 (**)	102.7 ± 12.2 (*)	40.5 ± 4.8 (**)
TRAN	5	166.1 ± 17.0 (**)	42.6 ± 6.6 (**)	40.4 ± 5.6 (**)
TRAN + SEHB	5 + 500	130.8 ± 9.1 (**)	48.7 ± 8.6 (**)	24.6 ± 3.4

*The results are expressed as mean ± s.d. Results with *p < 0.05 or ** p < 0.01 were considered significant compared to the Control group. ANOVA (one-way) followed by Dunnett post hoc test. Standard extract of H. brasiliense (SEHB), fluoxetine (FLU), GBR-12909 (GBR) and tranylcyprimine (TRAN).*

Open field test

SEHB has caused a decreasing dose-response effect in the motion activity. Only SEHB (150 mg/kg) has decreased the motion activity by 40% ($p < 0.01$). However, SEHB increased in a dose-dependent manner and statistically significant the rearing parameter using the dose 300 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.01$). When administered with control drugs, SEHB seems to give a low interaction at motion activity, but decreases the effect of GBR-12909 and tranylcypromine with no changes in fluoxetine effect when rearing was evaluated. Results are shown in Table 1.

Rota rod test

Treatment with different doses of SEHB did not produce significant statistical differences between control and treated groups on latent time. However, treatments display a dose response effect, reducing latent time.

DISCUSSION AND CONCLUSION

It has been widely accepted that affective states, such as mood, are mainly regulated by serotonin and nor epinephrine (17). Classical and atypical antidepressants have been designed to interfere with the action of these neurotransmitters (18; 19). Previous reports had shown that *H. brasiliense* dichloromethane and methanol extracts, as well as isolated compounds (xanthones and flavonoids) are able to inhibit MAO activity, with selectively to MAO_A (9). Tranylcypromine (a non-selectively IMAO) reduced the immobility time and its effect was increased when SEHB combined. The same may be observed with GBR-12909, which suggests a non-competitive synergistic action mechanism. Association of SEHB with fluoxetine displays an antagonistic effect, blocking fluoxetine effects. The open field test is a sensitive method to evaluate different types of drugs, dividing basically in 3 distinct behaviour as anxiolytic-like effects (characterized by increase in central arena locomotion without modification of other parameters), anxiogenic-like effects (which decreases motion, central motion and rearing) and stimulant-like effects (which result in an increase of motion) (20). Treatment with SEHB showed an anxiogenic-like effect at low dose (150 mg/kg) decreasing motion activity. SEHB has produced a dose dependent increase in rearing parameter. When SEHB was administered combined with GBR-12909 or tranylcypromine, a decrease in rearing was noticed. This effect was not observed when fluoxetine was administered combined with SEHB. The administration of 10 mg/kg of *H. perforatum* extract has decreased the immobility time in FST and motion activity in OFT, while it has increased rearing and exploratory behavior in OFT (21). Other *Hypericum* species have CNS activities, such as an infusion of *H. canariense*, *H. glandulosum*, *H. reflexum* or *H. grandifoliumi*, which decreases immobility time in FST with mice (22).

Chemical characterization and standardization of *H. brasiliense* extract displayed an important improvement to develop a new botanical agent with commercial viability to pharmaceutical companies. It is interesting to report that a naphthodianthrone called

hypericin, used as phytochemical marker in *Hypericum perforatum* extracts have phototoxic actions, and its was not founded in SEHB.

For the first time, the antidepressant activity of *Hypericum brasiliense* has been reported. This activity was suggested by the immobility time decrease without motion activity changes and increase of rearing. The *H. brasiliense* standard extract needs more studies to confirm its antidepressant properties, about its mechanism of action and toxicology.

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