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Pharmacological and neurochemical evidence for antidepressant-like effects of the herbal product Catuama

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Abstract

Catuama is a marketed herbal product currently used as a tonic, especially for the management of mental or physical fatigue. In the present study, we have shown pharmacological and neurochemical evidence for antidepressant-like actions of the product Catuama. Acute and chronic oral treatments with Catuama both resulted in a significant reduction of the immobility time in two models of depression in mice, the forced swimming and the tail suspension tests. Conversely, treatment with the same doses of Catuama did not significantly interfere with motor activity according to assessment in the open-field test. The antidepressant-like effects were comparable to those observed for classical antidepressant drugs. When assessed in vitro, Catuama inhibited, in a concentration-dependent manner, the synaptosomal uptake of noradrenaline and principally of serotonin and dopamine, in rat brain. Likewise, in vitro incubation of Catuama also resulted in a marked increase of the release of serotonin and dopamine in rat brain crude preparation of synaptosomal membranes. Finally, Catuama was found to be effective in interfering with the synaptosomal uptake of serotonin and dopamine following long-term oral treatment of rats. The present findings allow us to suggest that the herbal product Catuama might be useful for the clinical management of moderate and mild depressive states, alone or in association with current antidepressant drugs.

Keywords: Herbal product; Catuama; Depression; Neurotransmitter uptake and release

1. Introduction

The herbal product Catuama has been marketed in Brazil for more than 20 years and it is used as a tonic for the management of several disorders, including mental and physical fatigue, stress, and muscular asthenia. It is made up of the association of four hydroalcoholic extracts obtained from the following plants: *Trichilia catigua* (Meliaceae), *Paullinia cupana* (Sapindaceae), *Ptychopetalum olcaloides* (Olacaceae) and *Zinziber officinalis* (Zingiberaceae). The dried extract of Catuama contains 40.31% of *P. cupana*, 28.23% of *T. catigua*, 28.23% *P. olcaloides* and 3.26% of *Z. officinallis*.

Previous studies have demonstrated that Catuama presents relaxant actions on different vascular preparations obtained from rats, guinea pigs and rabbits. Evidence has suggested that the vasorelaxant actions of Catuama are largely dependent on the release of nitric oxide from endothelium (Cabrini and Calixto, 1997). In addition, it has been demonstrated that Catuama exerts antinociceptive actions, when administered by the oral route, in several models of chemical and thermal nociception (Vaz et al., 1997), via interaction with the nitric oxide pathway and the opioid system. More recently, Antunes et al. (2001) have shown that Catuama is capable of inducing relaxation of the rabbit corpus cavernosum in vitro, but in this case, its effects do not seem to rely on the production of nitric oxide.

Based on the aforementioned evidence and also on the indications of the herbal product Catuama, which include the control of symptoms associated with stressful and/or

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tensional conditions, it might be suggested that Catuama could be useful for the control of other central-nervous-system-related conditions, including mood disorders and moderate or mild depression states. In the present work, we have assessed the possible antidepressant-like effects of this product by means of in vivo and in vitro pharmacological and neurochemical procedures.

2. Methods

2.1. Animals

Nonfasted male Swiss mice (25–30 g; $n=66$) or Wistar rats (140–180 g; $n=70$) kept in controlled room temperature (22 ± 1 °C) under a 12:12 h light–dark cycle (lights on 0600 h) were used. The animals were acclimatized to the laboratory for at least 1 h and were used only once in each test. For the behavioural tests, animals were visually and acoustically isolated during the experimental sessions. Procedures were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983).

2.2. Forced swimming test

Experiments were performed according to the methodology originally described by Porsolt et al. (1977) with minor modifications (Rodrigues et al., 2002). For this purpose, mice were individually placed in cylindrical recipients (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C. The total immobility time was measured during a period of 6 min. Mice were considered immobile when they ceased struggling or remained floating in the water. Animals were treated with Catuama (150 to 300 mg/kg po) or saline (10 ml/kg po) 6 h beforehand. In other experiments, mice were subchronically treated with Catuama (200 mg/kg po) for 7 days. Separate groups of animals received the classical antidepressant imipramine (15 mg/kg ip, 30 min or 10 mg/kg ip, 7 days), which was used as a positive control. The time of treatment and the doses of Catuama were chosen on the basis of a previous study (Vaz et al., 1997).

2.3. Tail suspension test

Mice were treated with the herbal product Catuama (150–300 mg/kg po, 6 h), with imipramine (15 mg/kg ip, 6 h), or with saline (10 ml/kg po, 30 min) and the immobility time was quantified as previously (Steru et al., 1985; Rodrigues et al., 2002). Animals were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail. The time during which mice remained immobile was quantified during a period of 6 min.

2.4. Open-field test

To assess the possible effects of Catuama on motor activity, mice were evaluated in the open-field paradigm, according to the method characterised by Holland and Weldon (1968). Mice were individually placed in an acrylic box (40 × 60 × 50 cm) with the floor divided into 12 squares. The number of squares crossed with the four paws was registered during a period of 6 min. Animals were treated with Catuama (300 mg/kg) or with saline (10 ml/kg) given by the oral route 6 h before the experiments.

2.5. Synaptosomal neurotransmitter uptake

These series of experiments were performed with rats. The crude preparation of synaptosomal membranes was performed according to the technique described by Bennett et al. (1993) and modified by Chatterjee et al. (1998). Animals were sacrificed by decapitation and the striatum (for dopamine) or the cortices (for serotonin and noradrenaline) were dissected. Cortices and striatum were respectively homogenised in 15 or 10 ml of sucrose solution (0.32 M, 4 °C) and diluted in 10 ml of HEPES–Krebs buffer (composition in mM: NaCl 150, HEPES 10, KCl 6.2, Na_2HPO_4 1.2, $MgSO_4$ 1.2, glucose 10) containing pargylin (10 μM) and ascorbic acid (0.1%, pH 7.4). The homogenate was centrifuged at 750 × g for 10 min and the nuclear fraction was discarded. The supernatant was centrifuged at 17,400 × g for 20 min at 4 °C to obtain the synaptosomal fraction. Crude preparation of synaptosomal membranes were resuspended in HEPES–Krebs buffer and incubated (37 °C, 10 min) in the presence or absence of different concentrations of Catuama (10–1000 μg/ml). Fluoxetine (35 μg/ml), cocaine (3.4 μg/ml) or desipramine (30 μg/ml) were used as positive controls for the uptake of serotonin, noradrenaline and dopamine, respectively.

For the uptake analysis, crude preparation of synaptosomal membranes were incubated with [^{3}H]serotonin (2.9 nM), [^{3}H]dopamine (3 nM) or [^{3}H]noradrenaline (4 nM) at 37 °C, for 4 (serotonin and dopamine) or 10 min (noradrenaline). After the incubation period, the reaction was stopped by filtration (glass fibre filters, GF/B). The filters were placed in glass vials containing 4 ml of scintillation liquid. The radioactivity was measured following 12 h in a liquid scintillation counter (Packard, Tri-Carb 1600 TR). Nonspecific uptake was determined in parallel by the incubation of 1 mM of the unlabelled neurotransmitters. The incubation time points were selected with basis on previous studies, reflecting conditions where the uptake is linear (Vosmer et al., 1980; Richelson and Pfenning, 1984; Rothman et al., 1993; Wells et al., 1999).

In a separate group of experiments, to evaluate the effects of the long-term treatment with Catuama on the serotonin or dopamine uptake, rats were treated with Catuama (200 mg/kg po) or fluoxetine (10 mg/kg ip) once a day for 42 days. Control animals received saline (10 ml/kg po). At the end of

the treatment period, animals were sacrificed and the experiments were conducted as described before. All experiments were performed in triplicate and at least three animals were used per group.

2.6. Synaptosomal neurotransmitter release

The crude synaptosomal membrane preparation was assessed as described above using dissected cortices (for serotonin) and striatum (for dopamine) from rats. The synaptosomal fractions were incubated in HEPES–Krebs buffer (37 °C, 15 min) in the presence or absence of [^{3}H]serotonin (2.9 nM) or [^{3}H]dopamine (3 nM). Following the incubation period, the crude preparation of synaptosomal membranes were centrifuged at 12,000 × *g* for 30 s (three times) to eliminate the free radioactivity.

For the analysis of neurotransmitter release, crude preparation of synaptosomal membranes were incubated (37 °C, 4 min) in the presence or absence of different concentrations of Catuama (1–300 μg/ml). After the incubation, the crude preparation of synaptosomal membranes were centrifuged (12,000 × *g*, for 30 s) with the aim of separating the released monoamine. The supernatant was collected and placed in glass vials for posterior quantification. The pellets were suspended in cold buffer and transferred to other vials to quantify the monoamines contained in the crude preparation of synaptosomal membranes. Samples were mixed with scintillation liquid and radioactivity was counted as described before. Nonspecific release was determined by the incubation of parallel probes with 1 mM of the unlabelled neurotransmitters. The percentage of serotonin or dopamine release was determined by dividing the radioactivity present in the supernatant for the sum of the radioactivity present in the supernatant and in the pellet, multiplied by 100. Experiments were performed in triplicate and at least three animals were used per group.

2.7. Drugs

The herbal product Catuama was provided by Laboratório Catarinense, Joinville, SC, Brazil. The following drugs or reagents were used: ascorbic acid, desipramine, dopamine, imipramine, noradrenaline, pargylin, serotonin (all from Sigma, St. Louis, MO, USA). Fluoxetine (Novaquímica/SIGMA PHARMA, Brazil, Daforin). Cocaine, Tris, NaCl, KCl, $MgSO_4$, Na_2HPO_4, sucrose (Merck, Darmstadt, Germany). HEPES (GIBCO BRL, Gaithersburg, MD, USA). [^{3}H]serotonin (specific activity: 11.8 Ci/mmol), [^{3}H]dopamine (specific activity: 6.6 Ci/mmol), [^{3}H]noradrenaline (specific activity: 28 Ci/mmol) all from Amersham. All solutions were prepared just before use.

2.8. Statistical analysis

Results are presented as the mean ± S.E.M. of three to six experiments. The percentages of inhibition are reported as the mean ± S.E.M. of inhibitions obtained for each individual experiment. The IC_{50} or EC_{50} values (i.e., the concentrations of Catuama necessary to inhibit the uptake or to increase the release of neurotransmitters by 50%, respectively) are presented as geometric means accompanied by their respective 95% confidence limits. These values were determined by use of the least-squares method for individual experiments. Statistical comparison of data was performed by means of analysis of variance (ANOVA) followed by Dunnett's or Newmann–Keuls' test. *P* values less than .05 were considered significant.

3. Results

3.1. In vivo evidence of Catuama's antidepressant-like actions

The effects of treatment with Catuama were evaluated in two models of depression in mice. The acute treatment with Catuama (300 mg/kg po, 6 h prior) reduced, in a significant manner, the duration of immobility in the forced swimming test (34 ± 9%) (Fig. 1A). This schedule of treatment with Catuama also resulted in a significant inhibition of immobility time (45 ± 9%) according to evaluation in the tail suspension test (Fig. 1B). In both models of depression, the effects of Catuama were similar to those observed for the classical antidepressant imipramine (15 mg/kg ip, 30 min). The percentages of inhibition for imipramine were 39 ± 8% and 45 ± 9% in the forced swimming and tail suspension tests, respectively (Fig. 1A and B).

The results depicted in Fig. 2 demonstrate that the subchronic treatment with Catuama (200 mg/kg po) or with the classical antidepressant drug imipramine (10 mg/kg ip), once a day for 7 consecutive days, significantly decreased the duration of immobility in the forced swimming test. The inhibitions obtained were 37 ± 2% and 34 ± 5%, respectively.

Despite its antidepressant-like effects, Catuama (up to 300 mg/kg) failed to affect the motor activity of mice according to assessment in the open-field test (number of crossings: 91 ± 5% and 83 ± 4%, for control and treated animals, respectively; $n = 6$ per group). In addition, the treatment with Catuama once a day for 7 days (in doses up to 5000 mg/kg) did not evoke any toxic action, as indicated by the absence of effects on several behavioural and physiological parameters, including rectal temperature, general activity, sedation, tremors, alteration of muscular tonus, ptosis or abdominal constrictions.

3.2. Neurochemical evidence for antidepressant effects of Catuama

The results show that the herbal product Catuama (10–1000 μg/ml) reduced, in a significant and concentration-dependent manner, the synaptosomal uptake of [^{3}H]seroto-

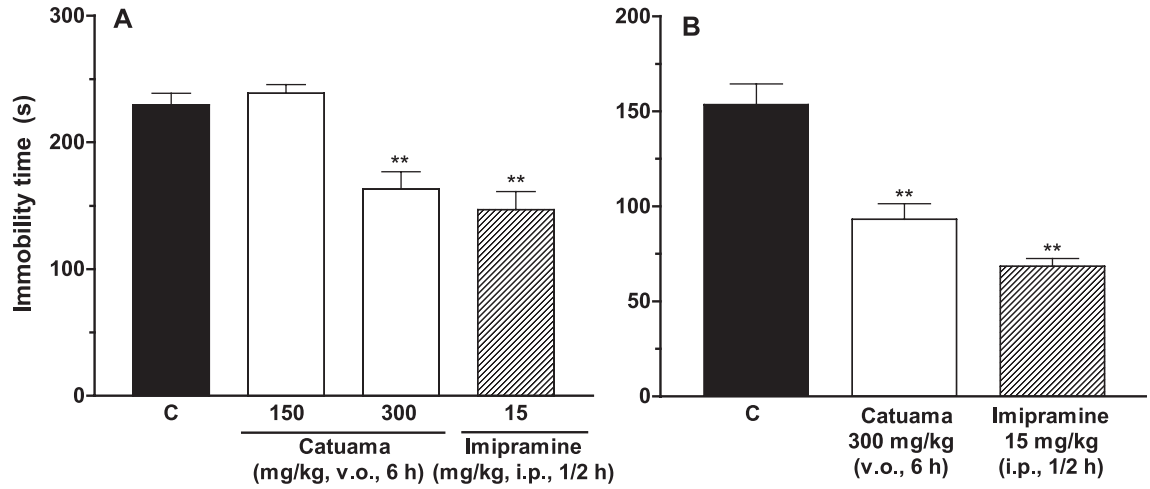

Fig. 1. Effect of treatment with the herbal product Catuama (150–300 mg/kg po, 6 h) or with imipramine (15 mg/kg ip, 15 min) on the forced swimming test (Panel A) or the tail suspension test (Panel B) in mice. Each column represents the mean ± S.E.M. of six animals. Significantly different from saline-treated animals, $^{**}P<.01$.

nin (Fig. 3A), [^{3}H]noradrenaline (Fig. 3B) or [^{3}H]dopamine (Fig. 3C) in synaptosomal preparations from rats. The maximal inhibitions obtained were 93 ± 1%, 58 ± 6% and 94 ± 1%, respectively. When analysed at the IC_{50} level, Catuama was about 9 and 18 times more potent in inhibiting the serotonin and the dopamine uptake, respectively, in relation to noradrenaline. The IC_{50} values accompanied by the 95% confidence limits were as follows: 80 (64–101), 39 (33–47) and 723 (551–949) μg/ml, respectively. The uptake of [^{3}H]serotonin, [^{3}H]dopamine and [^{3}H]noradrenaline was also significantly inhibited by fluoxetine (35 μg/ml), cocaine (3.4 μg/ml) and desipramine (30 μg/ml: 73 ± 5%, 73 ± 4% and 77 ± 4%, respectively; Fig. 3).

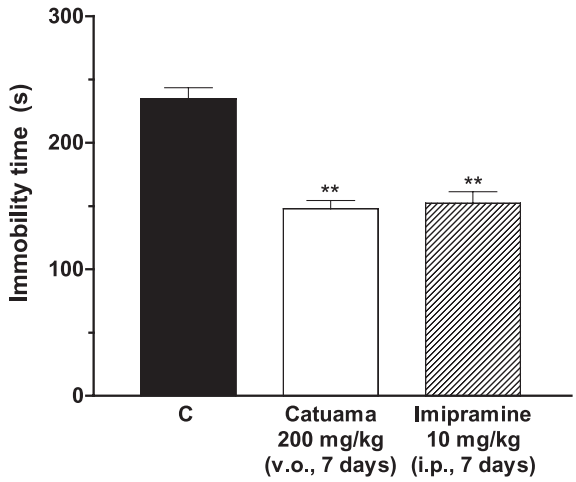

Fig. 2. Effect of subchronic treatment with the herbal product Catuama (200 mg/kg po) or with imipramine (10 mg/kg ip) during 7 days on the forced swimming test in mice. Each column represents the mean ± S.E.M. of six animals. Significantly different from saline-treated animals, $^{**}P<.01$.

Interestingly, the results presented in Fig. 4 indicate that the chronic treatment of rats with Catuama (200 mg/kg po) or with fluoxetine (10 mg/kg ip) once a day for 42 days resulted in a significant inhibition of both serotonin (Fig. 4A) and dopamine uptake (Fig. 4B). Long-term in vivo treatment with Catuama also inhibited serotonin uptake by 35 ± 12%, whereas dopamine uptake was decreased by 57 ± 3%. In contrast, fluoxetine treatment reduced serotonin uptake by 69 ± 2%, reducing dopamine uptake only by 28 ± 8%.

The neurochemical studies were extended by experiments with monoamine release, which demonstrated that in vitro incubation with Catuama (10–300 μg/ml) increased, concentration-dependently, the release of serotonin in synaptosomal fractions obtained from rats (Fig. 5A). The maximal response and the mean EC_{50} value for this effect was 116 ± 20% and 49 (21–114) μg/ml, respectively. In addition, the results demonstrate that Catuama (10–300 μg/ml) was more potent (about 2.8 times) and also more efficacious in increasing dopamine release, with a maximal response of 407 ± 68% and mean EC_{50} value of 17 (8–38) μg/ml (Fig. 5B).

4. Discussion

Depression constitutes the second-most common chronic condition in clinical practice, exceeded only by hypertension. Despite recent progress achieved in the development of clinically relevant antidepressant drugs in recent years, the currently available antidepressant therapy is not yet totally effective and it is associated with many undesirable collateral effects (Whooley and Simon, 2000; Nestler et al., 2002). In addition, only 60% of patients are responsive to the treatment with the available antidepressants (Moller and

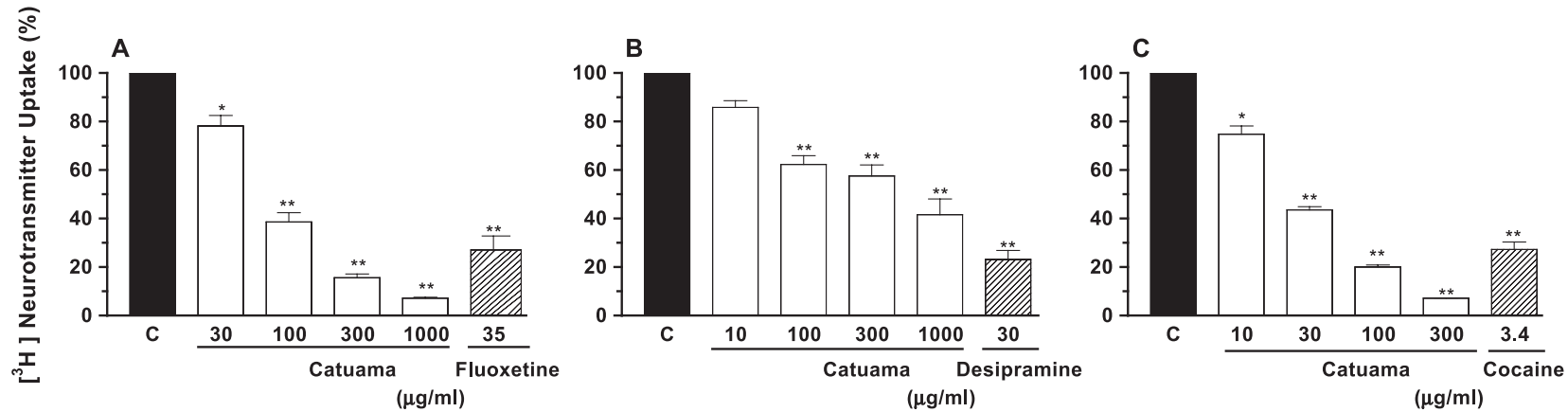

Fig. 3. In vitro effects of the herbal product Catuama (30–1000 μg/ml) on the synaptosomal uptake of [^{3}H]serotonin (Panel A), [^{3}H]noradrenaline (Panel B) or [^{3}H]dopamine (Panel C) in rats. Fluoxetine (35 μg/ml), desipramine (30 μg/ml) and cocaine (3.4 μg/ml) were used as positive control drugs. Each column represents the mean ± S.E.M. of three independent experiments performed in duplicate (percentage of specific uptake). Significantly different from control values, $*P<.05$; $**P<.01$.

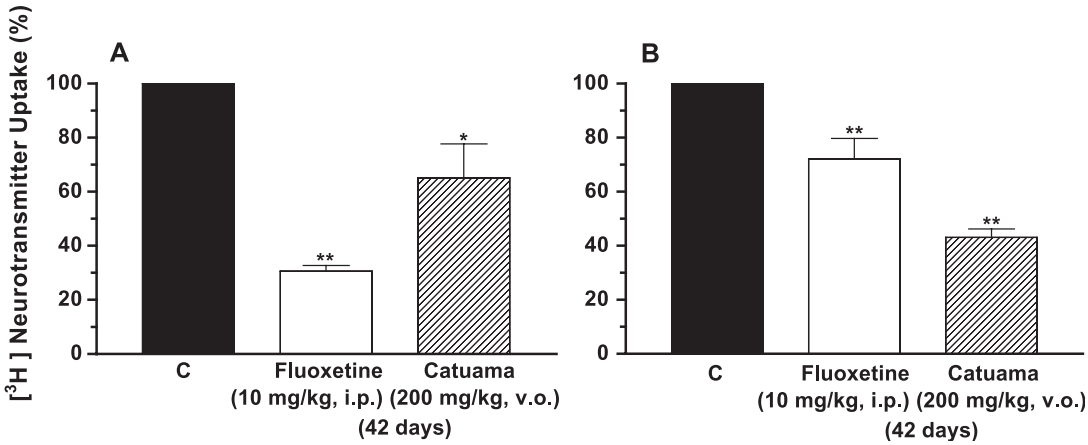

Fig. 4. Effects of chronic treatment with the herbal product Catuama (200 mg/kg po) during 42 days on the synaptosomal uptake of [^{3}H]serotonin (Panel A) or [^{3}H]dopamine (Panel B) in rats. Fluoxetine (10 mg/kg ip) was used as a positive control. Each column represents the mean ± S.E.M. of three to five independent experiments performed in duplicate (percentage of specific uptake). Significantly different from saline-treated animals, * $P<.05$; ** $P<.01$.

Volz, 1996; Gareri et al., 2000). For this reason, the search for new drugs for the control of the symptoms associated with depressive disorders is still desirable. In the present study, we have provided pharmacological and neurochemical evidence for Catuama's antidepressant-like activities.

Our results demonstrate that Catuama was consistently effective when evaluated in two classical models of depression in rodents, the forced swimming and the tail suspension tests. It is worth mentioning that the tests employed in this study are well characterized and validated for several distinct classes of drugs, including tricyclic and atypical antidepressants, and are good indicators for antidepressant actions in humans (Porsolt et al., 1977; Steru et al., 1985; Müller, 2003). In these models, both the acute and the subchronic treatment with Catuama resulted in a significant inhibition of the immobility time, with a profile comparable to that observed for the two classical antidepressant drugs imipramine and fluoxetine (present results and Rodrigues et al., 2002). In addition, the effects of Catuama were somewhat similar to those reported for the herbal drug *Hypericum perforatum* (St. John's wort) and its constituents (mainly hyperforin) in the forced swimming test (Chatterjee et al., 1998; Franklin and Cowen, 2001; Misane and Ogren, 2001; Roz et al., 2002; Müller, 2003).

As discussed before, the herbal product Catuama exerts antinociceptive effects according to evaluation in several models of chemical and thermal nociception (Vaz et al., 1997). In the present study, we have demonstrated that Catuama shows antidepressant-like effects when tested at the same doses and under the same schedules of treatment used previously. In fact, it has been widely reported that many classical antidepressant drugs present antinociceptive actions (Eschalier et al., 1992; Casas et al., 1995; Rodrigues-Filho and Takahashi, 1999).

It has also been reported that the acute treatment with some nonantidepressant drugs, including certain stimulants,

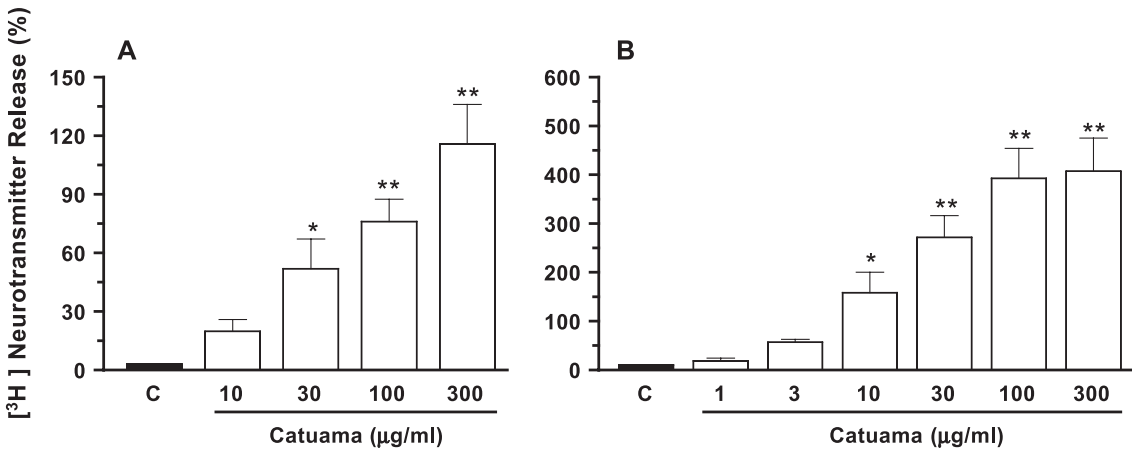

Fig. 5. In vitro effects of the herbal product Catuama (1–300 µg/ml) on the synaptosomal release of [^{3}H]serotonin (Panel A) or [^{3}H]dopamine (Panel B) in rats. Each column represents the mean ± S.E.M. of three independent experiments performed in duplicate (percentage of specific release). Significantly different from control values, * $P<.05$; ** $P<.01$.

convulsants and anticholinergics, among others, may reduce the duration of the immobility time in the forced swimming test, as they produce a general increase of the motor activity (Browne, 1979; Betin et al., 1982; Butterweck et al., 2003). According to this, one might suggest that the antidepressant-like effects of Catuama could be related with their stimulant actions. However, we discarded this possibility on the basis that results demonstrating that Catuama (in doses up to 300 mg/kg po) did not significantly interfere with ambulatory activity, when assessed in the open-field test.

Pharmacological evidence for the antidepressant effects of Catuama were extended by neurochemical studies, which clearly demonstrated that Catuama was capable of inhibiting, in a concentration-dependent fashion, the uptake of [^{3}H]serotonin, [^{3}H]noradrenaline and [^{3}H]dopamine. In these experiments, Catuama was about 9 and 18 times more potent in blocking the synaptosomal uptake of [^{3}H]serotonin and [^{3}H]dopamine, respectively. Interestingly, the in vivo long-term treatment with Catuama (for 42 days) markedly inhibited the synaptosomal uptake of [^{3}H]serotonin and more significantly of [^{3}H]dopamine in rat brains. Such findings suggest that the inhibition of the monoamine uptake, mainly of serotonin and dopamine, might account for Catuama's in vivo antidepressant actions.

Of great interest are the results showing that Catuama was not only effective in preventing the uptake of monoamines, but was also able to significantly increase the release of [^{3}H]serotonin and [^{3}H]dopamine in synaptosomal preparations obtained from rats. Moreover, Catuama was more potent in inhibiting the release of [^{3}H]dopamine (about 2.8 times at the EC_{50} level) in comparison to the release of [^{3}H]serotonin. The interference of Catuama on the uptake and/or release of monoamines may be related to several mechanisms including the interaction with vesicular monoamine transporters or even the vesicular storage dependent on the membrane pH gradients (Roz and Rehavi, 2003). Therefore, more studies are necessary to understand the precise mechanisms through which the herbal product Catuama interferes with monoamine uptake and release.

Although the classical antidepressant drugs are known to interfere mainly with the availability of serotonin and noradrenaline, several lines of evidence have recently suggested that some of their actions are associated with dopamine pathway modulation (for review, see D'Aquila et al., 2000). Such interference with the dopaminergic system could explain at least in part the acute effects of some of the antidepressants and also of Catuama. It has been demonstrated that *H. perforatum* extracts and hyperforin itself inhibit the synaptosomal uptake of serotonin, dopamine, noradrenaline, gamma-aminobutyric acid and L-glutamate with a very similar potency. In this regard, Catuama, *H. perforatum* extracts and hyperforin seem to be quite distinct from the tricyclic antidepressants, which interfere with the dopamine uptake only at very high concentrations (Müller, 2003). To what extent the ability of Catuama in increasing the levels of dopamine contributes to its in vivo antidepressant-like activity needs to be further investigated. It is of worth to remark that despite the effects of Catuama on the monoamine uptake/release processes, the chronic treatment with this product was not associated with any undesirable effect when assessed in a phase I clinical study (unpublished results).

Taken together, the present results present convincing pharmacological and neurochemical evidence supporting antidepressant-like actions for the herbal product Catuama and open up new possibilities for the use of Catuama in the treatment of mood disorders, such as mild and moderate states of depression.

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