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Effect of Semi-purified Constituent from Guaraná Seeds [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] on Performance of Rats in Elevated T maze

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Dissertação apresentada ao Programa de Pós-graduação em Ciências Farmacêuticas (produtos naturais e sintéticos biologicamente ativos), da Universidade Estadual de Maringá para obtenção do título de Mestre em Ciências Farmacêuticas.

Orientadora: Prof. Dra. Elisabeth Ap. Audi

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Dedico este trabalho

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RESUMO

O objetivo deste estudo foi investigar o efeito da administração crônica por gavagem do constituinte semi-purificado (EPA; 4, 8 ou 16 mg/kg) da semente de *Paullinia cupana* (guaraná) em ratos submetidos ao labirinto em T elevado (LTE), um modelo animal de ansiedade generalizada e transtorno do pânico e avaliar o envolvimento da neutransmissão serotoninérgica e dopaminérgica. O inibidor seletivo de recaptação de serotonina (5-HT; ISRS), paroxetina (3 mg/kg), foi usado como controle positivo. Para avaliar o possível envolvimento da neurotransmissão serotoninérgica e dopaminérgica nos efeitos do EPA no LTE, doses inefetivas de metergolina (antagonista 5-$HT_{2A/2C}$) ou sulpiride (antagonista dopaminérgico), foram administradas agudamente por via intraperitoneal juntamente com EPA e paroxetina. A atividade locomotora dos ratos foi avaliada através do teste do campo aberto após cada tratamento de drogas. EPA (8 e 16 mg/kg) ou paroxetina (3 mg/kg) aumentaram a latência de fuga do braço aberto no LTE, indicativo de efeito panicolítico comparados ao grupo controle. Metergolina, na sua mais alta dose (1, 2 e 3 mg/kg), mas não sulpiride (10, 20 e 40 mg/kg) produziu um efeito panicolítico no LTE. O efeito panicolítico produzido pelo EPA (8 mg/kg) foi bloqueado por ambos antagonistas, metergolina (2 mg/kg) e sulpiride (20 mg/kg), enquanto que o efeito panicolítico produzido pela paroxetina (3 mg/kg) foi bloqueado somente pela metergolina (2 mg/kg) no LTE. Estes resultados mostraram que o tratamento crônico com EPA e paroxetina produziu efeito panicolítico no LTE, e que o sistema de neurotransmissão serotoninérgica está envolvido no efeito de ambas as drogas, mas sistema de neutransmissão dopaminérgica está envolvido somente no efeito do EPA.

Palavras-chave: labirinto em T elevado, guaraná, *Paullinia cupana*, transtorno do pânico, serotonina

ABSTRACT

The purpose of this study was to investigate the effects of chronic administration by gavage of the semi-purified constituent (EPA; 4, 8, or 16 mg/kg) of *Paullinia cupana* (guaraná) seeds in rats submitted to the elevated T maze (ETM) model of generalized anxiety and panic disorders. The selective serotonin (5-HT) reuptake inhibitor (SSRI, 5-HT), paroxetine (3 mg/kg), was used as a positive control. To evaluate the possible involvement of serotonergic and dopaminergic neurotransmissions in the effects of the EPA on ETM, ineffective doses of metergoline ($5\text{-}HT_{2A/2C}$ antagonist receptor) or sulpiride (dopaminergic receptor antagonist), were administered acutely by the intraperitoneal route together with the EPA or paroxetine. The locomotion of the rats was assessed in a circular arena following each drug treatment. EPA (8 and 16 mg/kg) or paroxetine (3 mg/kg) increased the one way-escape latency from the open arm in the ETM, indicative of a panicolytic effect compared to their control group. Metergoline, in the higher dose (1, 2, and 3 mg/kg), but not of sulpiride (10, 20, and 40 mg/kg) produced a panicolytic effect in ETM. The panicolytic effect produced by EPA (8 mg/kg) was blocked by both metergoline (2 mg/kg) and sulpiride (20 mg/kg), whereas the panicolytic effect produced by paroxetine (3 mg/kg) was blocked only by metergoline (2 mg/kg) in the ETM. These results showed that chronic treatment with EPA produced a panicolytic effect in the ETM, and that the dopaminergic and the serotonergic neurotransmission systems are involved in this effect.

Key words: elevated T maze, guaraná, *Paullinia cupana,* panic disorder, serotonin

SUMÁRIO

Effect of Semi-purified Constituent from Guaraná Seeds [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] on Performance of Rats in Elevated T maze

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Abstract

The purpose of this study was to investigate the effects of chronic administration of the semi-purified constituent (PEA; 4, 8, or 16 mg/kg) of *Paullinia cupana* (guaraná) seeds in rats submitted to the elevated T maze (ETM) model of generalized anxiety and panic disorders. The selective serotonin (5-HT) reuptake inhibitor (SSRI), paroxetine (3 mg/kg), was used as a positive control. To evaluate the possible involvement of serotonergic and dopaminergic neurotransmissions in the effects of the PEA on ETM, ineffective doses of metergoline ($5\text{-HT}_{2A/2C}$ antagonist receptor) or sulpiride (dopaminergic receptor antagonist), were acutely administered together with the PEA or paroxetine. Locomotion of the rats was assessed in a circular arena following each drug treatment. PEA (8 and 16 mg/kg) or paroxetine (3 mg/kg) increased the one way-escape latency from the open arm in the ETM, indicative of a panicolytic effect compared to their control group. Metergoline, in the higher dose (1, 2, or 3 mg/kg), but not of sulpiride (10, 20, or 40 mg/kg) produced a panicolytic effect in ETM. The panicolytic effect produced by PEA (8 mg/kg) was blocked by both metergoline (2 mg/kg) and sulpiride (20 mg/kg), whereas the panicolytic effect produced by paroxetine (3 mg/kg) was blocked only by metergoline (2 mg/kg) in the ETM. These results showed that chronic treatment with PEA produced a panicolytic effect in the ETM, and that the dopaminergic and the serotonergic neurotransmission systems are involved in this effect.

Key words: elevated T maze, guaraná, *Paullinia cupana (*Sapindaceae*),* panic disorder, serotonin.

Introduction

Anxiety disorders, such as generalized anxiety disorder, panic disorder, obsessive-compulsive disorder, and phobias have the highest prevalence among psychiatric diseases [1]. Although often transient and moderate, these disorders may also be severe and resistant to treatment, causing high costs to the public health system [2,3]. They have a substantial negative impact on quality of life [4] with a loss in professional performance, reduction in work hours, difficulty of adaptation and interpersonal relationships [5,6], in addition to marital and financial difficulties [7].

Because of their prevalence and the degree of suffering that they cause [8], anxiety disorders are among the most common reasons for searching for complementary therapies [9] and self-medication with medicinal herbs [10]. Although the available pharmacological treatments are effective, they have many limitations. Antidepressants, selective serotonin reuptake inhibitors (SSRI), produce an initial exacerbation of anxiety symptoms, especially in panic disorder [11], and resistance in approximately 30% of patients [12], while benzodiazepines have a high incidence of dependency [13,14].

It is estimated that Brazil harbors the world's largest plant biodiversity [15], and *Paullinia cupana* (H.B.K. var. *sorbilis* (Mart.) Ducke) is a part of this vast biodiversity. *Paullinia cupana,* belonging to the family Sapindaceae and popularly known as guaraná, is grown mainly in the central Amazon basin [16], and its pharmacological actions have been a target of interest of pharmaceutical laboratories. Its seed contains high concentrations of xanthines (3.0-6.0%), which

include 1,3-caffeine (trimethylxanthine) and traces of theophylline and theobromine, as well as high concentrations of polyphenols or saponins (7%), which include catechins, epicatechins, and other condensed tannins [16,17].

The wide use of extracts from seeds and roots of guaraná is due to their stimulant effects on the central nervous system [17]. The extract is used as an anorectic, a nootropic producing improvements in cognitive ability and memory, and an aphrodisiac [18,19,16]. Different pre-clinical and clinical studies have confirmed the popular use of guaraná seed extract to improve memory performance [20,21].

A semi-purified fraction obtained from an extract of guaraná seeds, termed purified extract A (PEA) (University of Maringá (UEM) patent applied for) improved performance and memory speed [22] and produced antidepressant-like effects in rats [23]. These effects were similar to those produced by the tricyclic antidepressant imipramine, but not by equipotent doses of caffeine, suggesting that other active substances present in the extract and fractions are responsible for these effects. Additionally, clinical studies have shown that guaraná improves the mood in healthy volunteers [24].

The aim of this study was to assess the anxiolytic and/or panicolytic effect of the PEA of guaraná on rats subjected to the elevated T-maze (ETM) test. To evaluate the mechanisms involved in the effects produced by PEA, the serotonergic (metergoline) or the dopaminergic (sulpiride) antagonists were used in combination with PEA in the ETM.

Materials and Methods

Plant material

The seeds of *Paullinia cupana* var. *sorbilis* (Mart.) Ducke (Sapindaceae) called guaraná were collected in the Alta Floresta region, state of Mato Grosso, Brazil. The samples were dried and provided by Mr. José Augusto de Souza. The dried seeds were pulverized in a hammer mill (Tigre ASN-5). A voucher plant specimen (#HUEM9065) was deposited with the Herbarium of the State University of Maringá (HUEM). The species was identified by Dr. Cássia Mônica Sakuragui.

Extracts

An extract was prepared from ground guaraná seeds (1000 g) with the extractor liquid acetone:water (7:3; v/v) by turbolise, and after removal of the organic solvent, the remaining solid material was lyophilized (EBPC – patent pending PI0006638-9). The semipurified, lyophilized extracts were obtained from the EBPC: 158 g of the lyophilized extract was partitioned with ethyl acetate (10x, 5 L), resulting in an ethyl-acetate fraction (PEA: 44 g) (patent pending PI0006638-9)**.** The PEA was solubilized in distilled water immediately before administration. Spectrophotometric analyses detected the proportions of 34.95±0.99% (RSD%=2.83) and 17.53±0.37% (RSD%=2.09) to the caffeine and tannin total respectively, for semipurified fraction (PEA). These results were obtained after analytic development method using UV-VIS spectrophotometry [25].

Sample preparation

The PEA fraction was extracted with solid phase cartridge (Phenomenex® Strata C18-E) in a methanol:water (10:90; v/v) to 80 µg/mL. The extracts were filtered (0.45 µm, Millipore®) prior to injection on HPLC.

HPLC apparatus and operating conditions

The experiments were performed in a Thermo® Finnigan Surveyor HPLC system coupled with a Thermo® UV/VIS plus detector and a injecting valve fitted with a 20 µL loop. Data acquisition was performed with the ChromQuest® 4.2 software. A Phenomenex® Synergi Polar – RP 80A (250 X 460 mm, 4 µm) and guard column (Analytical Guard Cartridge System KJO-4282) was used in all experiments. The water obtained from Milli-Q Gradient®. The mobile phase consisted of Phase A: methanol:acetonitrile (25:75; v/v) TFA 0,05% previously filtered through a 0.45 mm filter (Millippore) and Phase B: water TFA 0,05%, All solvents were degassed using an ultrasonic bath. The gradient system consisted of 0-20 min, 20 – 26% Phase A; flow-rate 0.9 mL/min; UV detection at 210 nm, and ambient column temperature.

Drugs

PEA, Paroxetine (positive control; IPCA Laborat, India), metergoline (SIGMA; 5-$HT_{2A/2C}$ receptor antagonist) and (-) sulpiride (SIGMA; non-selective

dopaminergic receptor antagonist) were solubilized in saline (0.9% NaCl) containing 2% Tween 80. The control group was treated with the vehicle (0.9% NaCl plus 2% Tween 80).

Animals

Male Wistar rats (55 days old, 230-250 g) housed 5 per cage at constant room temperature (22-23 °C) under a 12-h light-dark cycle with free access to food and water were used in the experiments. The experiments were performed between 13:00h and 18:00h. The experimental procedures adopted were approved by the UEM Ethics Committee (053/2008), and followed the recommended guidelines for Biomedical Research Involving Animals (CIMS), Geneva, 1985.

Apparatus

The ETM was constructed of wood and had three arms of equal dimensions (60 cm x 12 cm). One arm, enclosed by 40-cm-high walls, was perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a 1-cm-high Plexiglas rim. The entire apparatus was 60 cm above the floor. Locomotion was measured in a circular wooden arena, 70 cm diameter, with 30-cm-high walls. Luminosity at the level of the maze arms or at the center of the circular arena was 60 lux.

Behavioral Tests

Procedure

One day before the test each animal was pre-exposed to one of the open arms of the ETM for 30 min. A wood barrier mounted on the border of the maze central area and the arm's proximal end isolated this arm from the rest of the T maze. It has been shown that this pre-exposure to the open arm renders the escape task more sensitive to the effects of antipanic drugs, because it shortens the latencies of withdrawal from the open arm during the test [26]. The ETM test was performed 24 h later.

The test in the ETM was initiated by the inhibitory avoidance task. To this end, each animal was placed at the distal end of the enclosed arm of the ETM facing the intersection of the arms. The time taken by the rat to leave this arm with all four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30 s intervals. Following avoidance trials (30 s), rats were placed at the end of the same, previously experienced open arm, and the latency to leave this arm with all four paws was recorded for three consecutive trials (one-way escape 1, 2, and 3) at 30 s intertrial intervals. A cutoff time of 300 s was established for the avoidance and escape latencies. Thirty seconds after being tested in the ETM, each animal was placed for 5 min in the circular arena for the evaluation of locomotion.

The total distance traveled was analyzed by a video tracking system (Ethovision; Noldus, Holland).

Experiment 1

To determine the dose-response curve of the PEA, the animals were treated for 24 days with paroxetine (3 mg/kg), PEA (4, 8, and 16 mg/kg) or the vehicle by gavage (i.g.). In 23 days of treatment, the pre-test was performed where each animal was confined for 30 min in one of the open arms of the ETM, and only after the pre-test the animals received (drugs or vehicle) chronic i.g. treatment. In 24 days of treatment, the animals were treated with the vehicle by intraperitoneal (i.p.) route, and after 5 min received paroxetine, PEA or vehicle by i.g. route. After 60 min of the last treatment, the animals were submitted to behavioral tests.

To determine the dose-response curve of metergoline (1, 2, or 3 mg/kg) or sulpiride (10, 20, or 30 mg/kg), the animals were treated for 24 days with the vehicle by the i.g. route. In 23 days of treatment, the pre-test was performed as described above, and only after the pre-test the animals received the vehicle i.g. treatment. In 24 days of treatment, the animals were treated with the antagonists or vehicle by the i.p. route, and after 5 min received the vehicle by the i.g. route. After 60 min of the last treatment, they were submitted to the behavioral tests.

Experiment 2

In the studies of association of metergoline or sulpiride with PEA or paroxetine, the animals were treated for 24 days with paroxetine (3 mg/kg), PEA (8mg/kg), or vehicle (i.g.). In 23 days of treatment, the pre-test was performed as described above, and only after the pre-test the animals received (drugs or

vehicle) chronic i.g. treatment. In 24 days of treatment, animals were treated by the i.p. route with the vehicle or antagonists and after 5 min received paroxetine (3 mg/kg), PEA (8 mg/kg) or vehicle by the i.g. route. After 60 min, behavioral tests were performed.

Statistical analysis

Repeated-measure analysis of variance (RMANOVA) was used to analyze both avoidance and escape data. The systemic treatments was considered the independent factors and tests (the baseline, avoidance 1 and 2, or leakage 1-3) as repeated measures. When appropriate, one-way ANOVA followed by the *post-hoc* Duncan's multiple comparison test, was used. Locomotion data were analyzed by one-way ANOVA followed by the *post-hoc* Duncan's multiple comparison test. Differences between groups were considered significant if $P<0.05$.

Results

Chromatogram of a 80 µg/mL extracts is seen in figure 1. The results indicated the principal components of the PEA fraction, and it have about 27% of catechin (peak 2), 38% of epicatechin (peak 4) and 27% of caffeine (peak 5).

Experiment 1

Figure 2 illustrate the effects of the administration of the vehicle (acute, i.p.) in rats chronically treated by i.g. route with the vehicle (control group), paroxetine (3 mg/kg), or PEA (4, 8, or 16 mg/kg) in the ETM. RMANOVA indicated for inhibitory avoidance a significant main effect of trial [$F_{(2.120)}$=68.37, p<0.001], but no significant effect of treatment [$F_{(4.60)}$=0,68, p=0.60], nor a significant treatment X trial interaction [$F_{(8.120)}$=1.32, p=0.23]. For escape, RMANOVA showed a significant main effect of treatment [$F_{(4.60)}$=5.79, p<0.001], but no significant effect of trial [$F_{(2.120)}$=1.85, p=0.16], nor a significant treatment X trial interaction [$F_{(8.120)}$=0.91, p=0.51]. *Post-hoc* comparisons showed that PEA (8 and 16 mg/kg) increased escape 2 (*p<0.05) and 3 (**p<0.01) latencies, respectively, as well as paroxetine (3.0 mg/kg) significantly increased the escape 2 (**p<0.01) and 3 (*p<0.05) latencies compared to the control group, indicating a panicolytic effect.

Figure 3 (A and C) illustrates the results observed with acute administration (i.p.) of the vehicle or metergoline (1, 2, or 3 mg/kg) in rats chronically treated with vehicle by i.g. route. RMANOVA for inhibitory avoidance (3A) showed a significant

main effect of trial [$F_{(2.54)}$=17.53, p<0.001] but no significant effect of treatment [$F_{(3.27)}$=1.07, p=0.37], nor a significant treatment X trial interaction [$F_{(6.54)}$=0.93, p=0,48]. For escape (3C), RMANOVA showed a significant main effect of treatment [$F_{(3.27)}$=4.01, p<0.05], but no significant effect of trial [$F_{(2.54)}$=0.38, p=0.68], nor a significant treatment X trial interaction [$F_{(6.54)}$=0.63, p=0.70]. *Post-hoc* comparisons showed that metergoline (3.0 mg/kg) significantly increased the escape 1 (**p<0.01) and 3 (*p<0.05) latencies compared to the control group, indicating a panicolytic effect.

Figure 3 (B and D) illustrates the results produced by acute administration (i.p.) of the vehicle or sulpiride (10, 20, and 40 mg/kg) in rats chronically treated with vehicle by i.g. route. RMANOVA for inhibitory avoidance (3B) showed a significant main effect of trial [$F_{(2.56)}$=28.93, p<0.001] but no significant effect of treatment [$F_{(3.28)}$=0.75, p=0.52], nor a significant treatment X trial interaction [$F_{(6.56)}$=1.11, p=0.36]. For escape (3D), RMANOVA showed no significant effect of trial [$F_{(2.56)}$=2.73, p=0.07], treatment [$F_{(3.28)}$=0.92, p=0.43], nor a significant treatment X trial interaction [$F_{(6.56)}$=0.14, p=0.98].

Experiment 2

Figure 4 (A and C) illustrates the results of the combination (acute, i.p.) of vehicle or metergoline (2 mg/kg) in chronically treated rats by i.g. route with the vehicle, paroxetine (3 mg/kg) or PEA (8 mg/kg) in the elevated T maze. For inhibitory avoidance (4A), RMANOVA showed a significant main effect of trial

[$F_{(2.116)}$=32.85, p<0.001], but no significant effect of treatment [$F_{(5.58)}$=1.16, p=0.34], nor a significant treatment X trial interaction [$F_{(10.116)}$=1.00, p=0.43]. For escape (4C), RMANOVA showed a significant main effect of treatment [$F_{(5.58)}$=7.20, p<0.001], but no significant effect of trial [$F_{(2.116)}$=1.37, p=0.25], nor a significant treatment X trial interaction [$F_{(10.116)}$=1.07, p=0.38]. *Post-hoc* comparisons showed that paroxetine (3.0 mg/kg) significantly increased the escape 1 (*p<0.05), 2 and 3 (**p<0.01) latencies, and PEA (8 mg/kg) increased escape 2 and 3 (**p<0.01) latencies compared to the control group, indicating a panicolytic effect. Metergoline (2 mg/kg) blocked the panicolytic effect produced by paroxetine, shown by a significant difference in the escape 1, 2 and 3 ($^{\#}p \leq 0.05$) latencies for MET + PAR compared to the VEH + PAR group. Also, metergoline (2 mg/kg) blocked the panicolytic effect shown for the PEA, as shown by significant difference in the escape 2 and 3 ($^{+}p<0.05$) latencies for MET + PEA compared to the VEH + PEA group. These results indicate that serotonergic neurotransmission is involved in the panicolytic effect of paroxetine and PEA in the ETM.

Figure 4 (B and D) illustrates the results of association of (acute, i.p.) sulpiride (20 mg/kg) in chronically treated rats by i.g. route with the vehicle, paroxetine (3 mg/kg), or PEA (8 mg/kg) on ETM. For inhibitory avoidance (4B), RMANOVA showed a significant main effect of trial [$F_{(2.108)}$=46.55, p<0.001], but no significant effect of treatment [$F_{(5.54)}$=1.17, p=0.33], nor a significant treatment X trial interaction [$F_{(10.108)}$=1.13, p=0.34]. For escape (4D), RMANOVA showed a significant main effect of treatment [$F_{(5.54)}$=3.10, p<0.05], but no significant effect of trial [$F_{(2.108)}$=2.72, p=0.07], nor a significant treatment X trial interaction

[$F_{(10.108)}$=1.67, p=0.09]. *Post-hoc* comparisons showed that paroxetine (3 mg/kg) and PEA (8 mg/kg) significantly increased the escape 2 (*p<0.05) latency compared to the control group, indicating a panicolytic effect. Sulpiride blocked the panicolytic effect of PEA on escape 2 ($^{+}$p<0.05), as shown by a siginificant difference of SUL+PEA compared VEH+PEA indicating the involvement of dopaminergic neurotransmission in the action mechanism of PEA.

The table 1 shows the distance traveled in meters in the circular arena. One-way ANOVA did not indicate significant changes in the distance traveled under the different treatments, compared to the control group.

Discussion

This is the first study evaluating the effect of PEA on rats in the elevated T maze, an animal model developed to assess defensive behaviors that have been related to specific subtypes of anxiety disorders, the generalized anxiety disorder and the panic disorder.

The data obtained showed that chronic treatment of PEA increased escape latency, without affecting basal and inhibitory avoidance latency, or locomotion in the circular arena, as well as paroxetine. Our results showed that PEA had a selective panicolytic effect on rats in the ETM after chronic treatment. The some PEA fraction of guarana produced antidepressant-like effect in the FST [23] and the principal components of the PEA fraction consisting in 65% of catechin and epicathechin.

Accordingly, polyphenols and tannins have antioxidant activity [27-29], and active substances may be responsible for the improved cognition produced by *Panax ginseng* [30] and red ginseng [31].

Antidepressant drugs of different classes, including tricycles, MAO inhibitors or SSRI, have been successfully used for the treatment of subtypes of anxiety disorder, including GAD [32,33], PD [34], obsessive-compulsive disorder [35], social anxiety disorder [36] and post-traumatic stress disorder [37], in addition to depressive disorders [38].

The effective doses of PEA (8 mg/kg) or paroxetine (3 mg/kg) were chosen to combine with ineffective doses of metergoline or sulpiride, the serotonergic or dopaminergic antagonists, respectively.

Metergoline as well as sulpiride blocked the panicolytic effect produced by PEA in the elevated T maze. As expected, the panicolytic effect of the SSRI, paroxetine, was blocked only by metergoline. These results show that both serotonergic and dopaminergic neurotransmission systems are involved in the panicolytic effect of PEA.

The paroxetine was choice as positive control in this study because it is the first SSRI approved by FDA to treatment of panic disorder [39] and many studies show its effectiveness and high tolerability in this pathology [40].

Although the SSRIs are considered the first-choice treatment in panic disorder, dopamine is an important neurotransmitter involved in the etiology and treatment of anxiety disorders and depression [41-43].

The blockage of the panicolytic effect of paroxetine by metergoline but not by sulpiride is in accordance with the expected results for an SSRI.

In conclusion, these results demonstrated that the PEA is active orally and produces a panicolytic effect on rats in the ETM. The serotonergic and dopaminergic neurotransmission systems are involved in this effect caused by PEA. Our study suggests that the PEA may be a useful drug in the treatment of mood disorders such as panic disorder.

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Legends for Figures

Figure 1. Chromatogram of PEA fraction with catechin (t_r=8.5 min; 2), epicatechin (t_r=10.1 min; 4) and caffeine (t_r=11.1 min; 5) at 210 nm.

Figure 2 – Effects (mean ± SEM) of chronic administration of control, paroxetine, or PEA on inhibitory avoidance and one-way escape latencies on elevated T maze (n=9-15). *P<0.05, **P<0.01 compared to the control group.

Figure 3 – Effects (mean ± SEM) of i.p. acute administration of metergoline (A,C) or sulpiride (B,D) in chronically treated rats with vehicle (i.g.) on inhibitory avoidance (upper panel) and escape (lower panel) latencies in elevated T maze (n=5-9). *P<0.05, **P<0.01 compared to the control group.

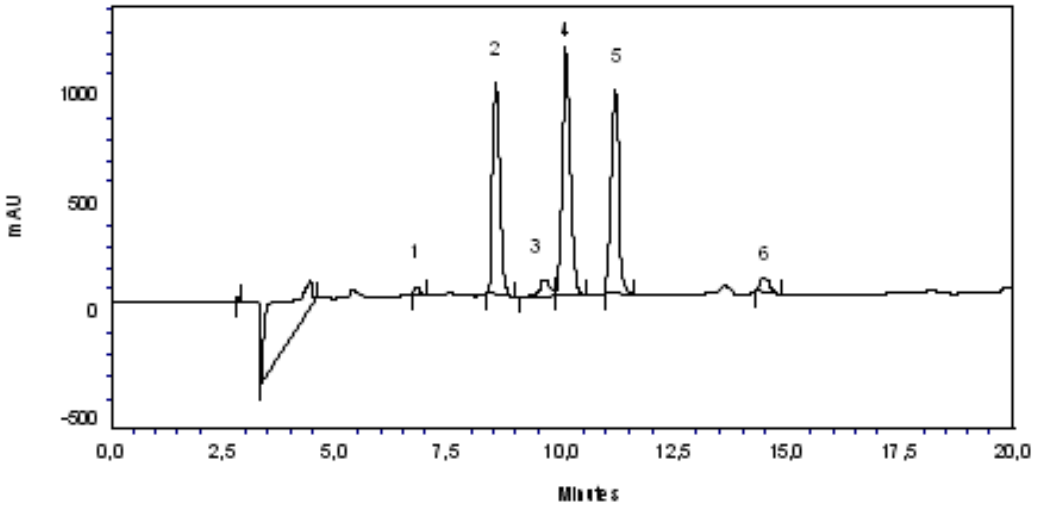
Figure 4 – Effects (mean ± SEM) of i.p. acute administration of metergoline or sulpiride in chronically treated rats (i.g.) with vehicle, paroxetine (3 mg/kg), or PEA (8 mg/kg) on inhibitory avoidance (upper panel) and one-way escape (lower panel) latencies in the elevated T maze. N =7-13). *****P<0.05, **P<0.01 compared to the control group. $^{\#}$P<0.05 compared to the VEH + PAR group. $^{+}$P<0.05 compared to the VEH + PEA group.

Table 1. Distance in meters travelled in the circular arena

Drug (mg/kg)	Distance Travelled (m)
Control	18.85±1.01
Vehicle + Paroxetine (3)	19.92±1.01
Vehicle + PEA (4)	15.71±1.13
Vehicle + PEA (8)	16.41±1.30
Vehicle + PEA (16)	19.73±1.04
Control	16.42±1.17
Metergoline (1) + Vehicle	17.67±1.48
Metergoline (2) + Vehicle	15.92±1.10
Metergoline (3) + Vehicle	15.85±1.10
Control	16.65±1.87
Sulpiride (10) + Vehicle	19.25±1.87
Sulpiride (20) + Vehicle	16.54±1.87
Sulpiride (40) + Vehicle	13.74±1.87
Control	17.62±1.02
Vehicle + Paroxetine (3)	19.00±1.02
Vehicle + PEA (8)	15.75±1.02
Metergoline (2) + Vehicle	15.92±1.23
Metergoline (2) + Paroxetine (3)	15.51±1.31
Metergoline (2) + PEA (8)	16.60±1.31
Sulpiride (20) + Vehicle	16.54±1.20
Sulpiride (20) + Paroxetine (3)	17.36±1.20
Sulpiride (20) + PEA (8)	17.68±1.20

Data are means ± SEM. N = 9-15. $p>0.05$ compared to control groups.

Figure 1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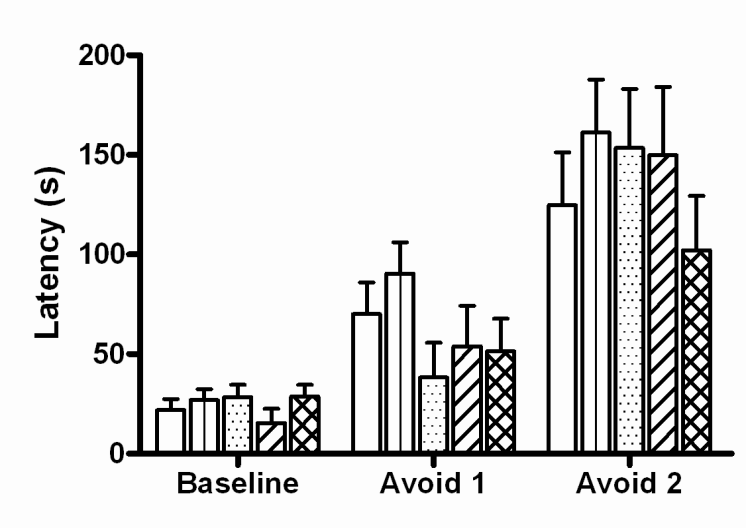
Minutes

Figure 2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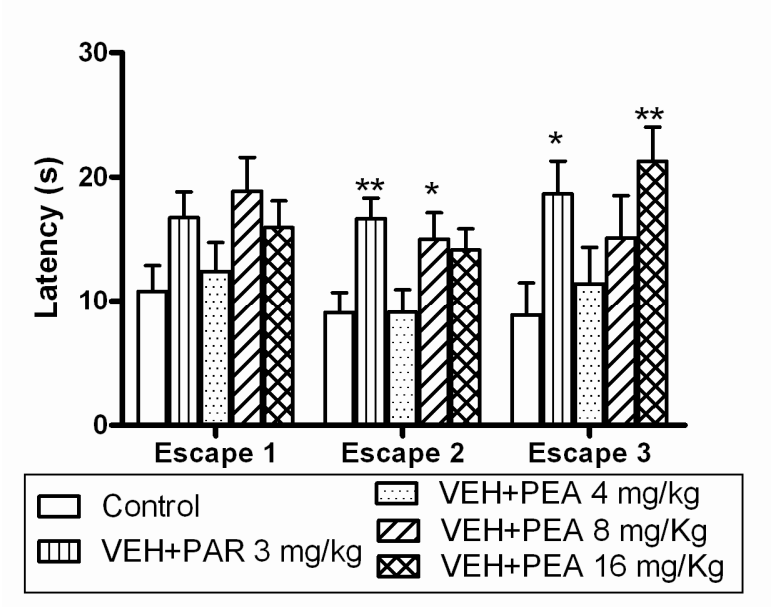
Avoid 2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Escape 1
Escape 2
Escape 3
Control
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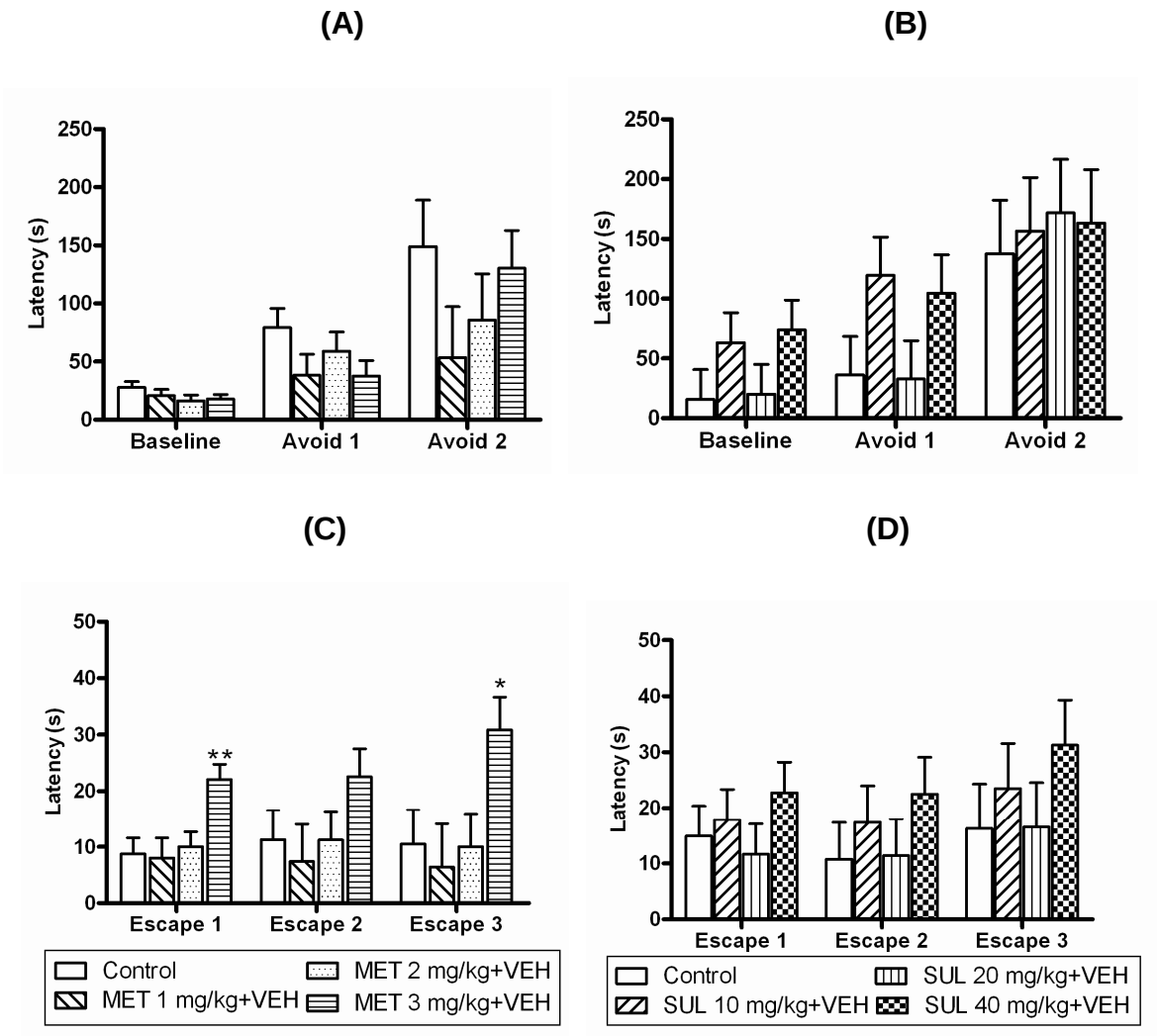
VEH+PEA 8 mg/Kg
VEH+PEA 16 mg/Kg

Figure 3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(B)
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Latency (s)
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200
150
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0
Baseline
Avoid 1
Avoid 2
50
40
30
20
10
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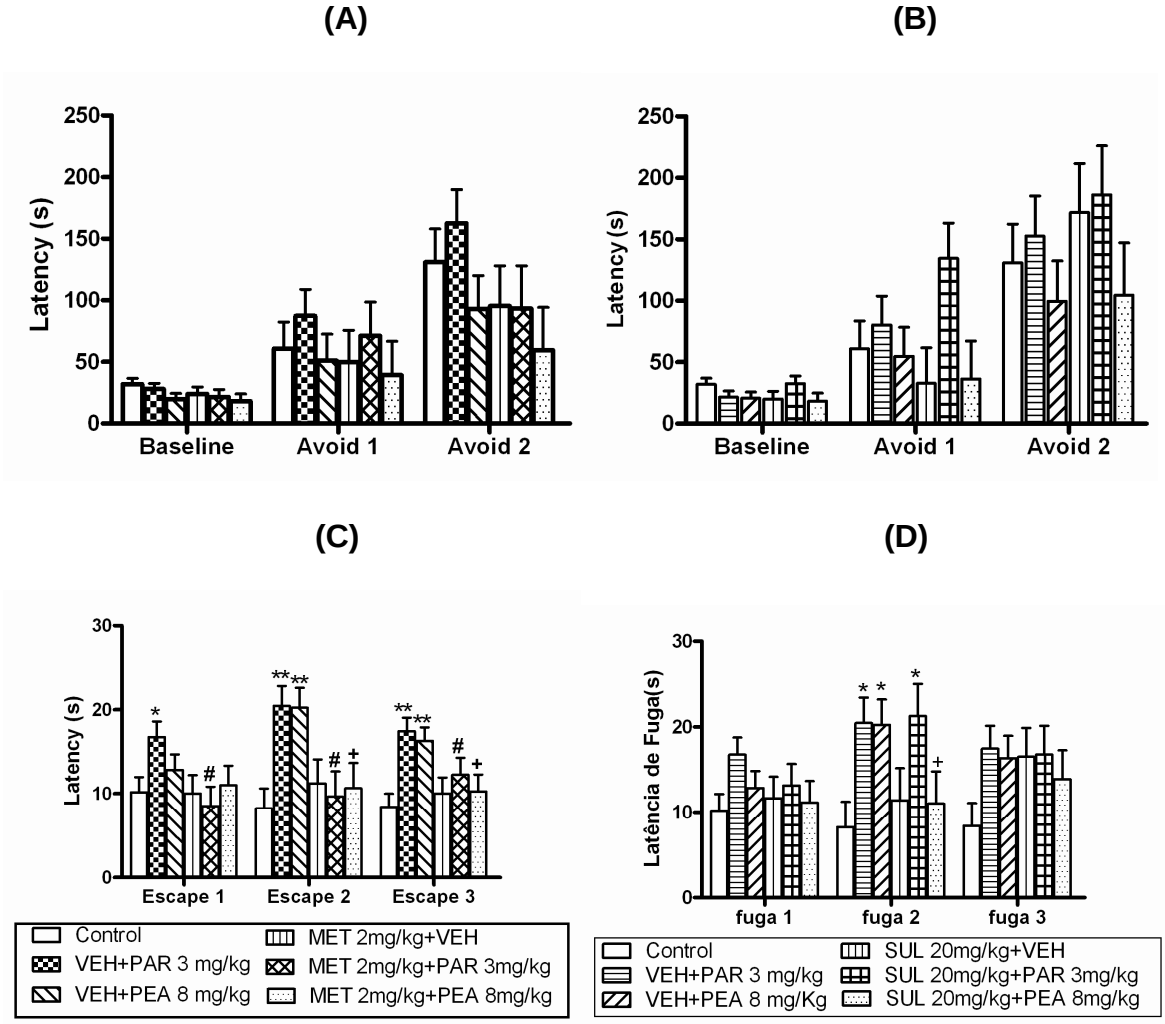
*
Escape 1
Escape 2
Escape 3
Control
MET 1 mg/kg+VEH
MET 2 mg/kg+VEH
MET 3 mg/kg+VEH
SUL 10 mg/kg+VEH
SUL 20 mg/kg+VEH
SUL 40 mg/kg+VEH

Figure 4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Latency (s)
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Baseline
Avoid 1
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(C)
(D)
Escape 1
Escape 2
Escape 3
Latência de Fuga(s)
fuga 1
fuga 2
fuga 3
Control
VEH+PAR 3 mg/kg
VEH+PEA 8 mg/kg
MET 2mg/kg+VEH
MET 2mg/kg+PAR 3mg/kg
MET 2mg/kg+PEA 8mg/kg
VEH+PEA 8 mg/Kg
SUL 20mg/kg+VEH
SUL 20mg/kg+PAR 3mg/kg
SUL 20mg/kg+PEA 8mg/kg