SHORT COMMUNICATION

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Anxiolytic effects of a passion flower (*Passiflora incarnata* L.) extract in the elevated plus maze in mice

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The purpose of this study was to characterize the putative anxiolytic-like activity of an ethanolic extract prepared from passion flower (PF) (*Passiflora incarnata* L.) using the elevated plus maze (EPM) in mice. The mice were either treated orally with three different concentrations of the PF extract or the positive control diazepam. The number of entries in the open arms was significantly increased after administration of diazepam compared to the control. PF extract showed a significant increase in number of open arm entries at a concentration of 375 mg/kg, whereas no activity was observed in 150 and 600 mg/kg, respectively, indicating an U-shaped dose response curve. In conclusion, using the EPM we were able to detect putative anxiolytic effects of a *Passiflora incarnata* extract in mice.

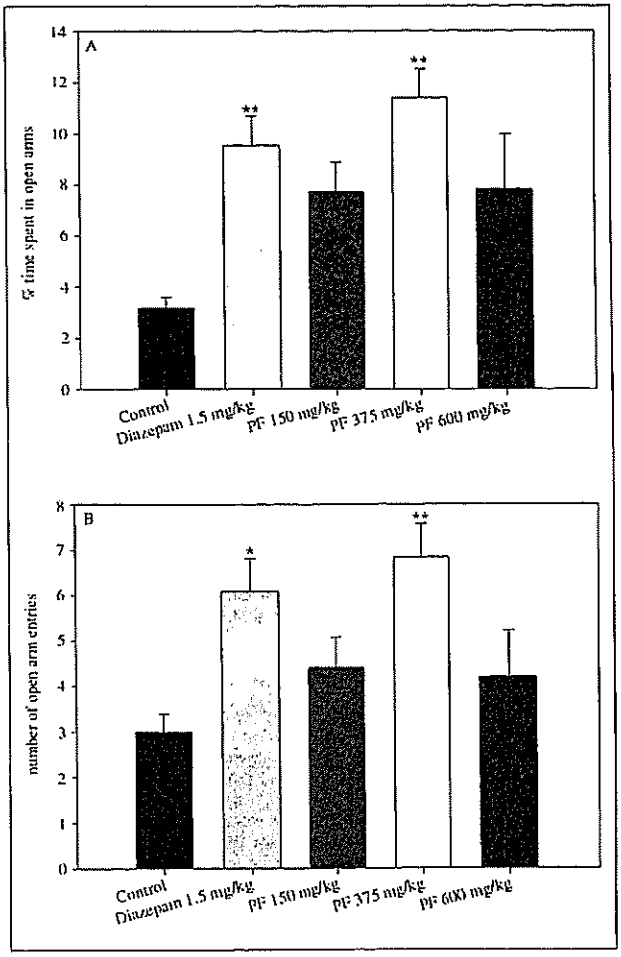

Fig.: Anxiolytic effects of passion flower (PF) extract (150, 375, and 600 mg/kg, p.o.) expressed by the percentage time spent in open arms (A), and number of open arm entries (B) in the EPM in mice. Results are expressed as MEAN ± S.E.M, n = 10 each group; ** $p < 0.01$ versus control; * $p < 0.05$ versus control, ANOVA with Dunnett's post-hoc test

The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans (Dawson and Tricklebank 1995). An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in open arms of the EPM. In the present study, oral administration of the tested PF extract induced an anxiolytic-like effect in mice, since it increased the number of entries and the time spent in open arms in the EPM test.

One-Way Analysis of Variance revealed a significant increase of percentage time spent in the open arms after administration the positive controls diazepam (9.5 ± 21.1, $p < 0.01$) compared to control (3.1 ± 0.4). PF extract showed significant activities in the percentage time spent in open arms in a concentration of 375 mg/kg (11.4 ± 1.1, $p < 0.01$) but not at 150 mg/kg (7.7 ± 1.1), and 600 mg/kg (7.8 ± 2.9) (Fig. A), indicating an inverted-U-shaped dose response curve. The occurrences of U-shaped dose-responses are a widely and independently observed phenomena (Calabrese and Baldwin 2001). Yet, despite the widespread nature of their occurrence, little attempt has been made to assess U-shaped dose-responses as integrative phenomena. Instead, they are regarded as a string of apparently reproducible, but biologically unrelated, responses (Calabrese and Baldwin 2001). However, this suggests that the widespread occurrence of these U-shaped dose-responses might be examples of biological optimization processes (Calabrese and Baldwin 2001; Calabrese et al. 1999).

The number of entries in the open arms was significantly increased after administration of diazepam (6.1 ± 0.7, $p < 0.05$) compared to the control (3.0 ± 0.3). PF extract showed a significant increase in number of open arm entries at a concentration of 375 mg/kg (6.8 ± 0.7, $p < 0.01$) (Fig. B).

A sedative or stimulatory effect of each of the compounds could be excluded as well, since none of the compounds had an influence on the total distance that the animals covered during the observation period (data not shown).

Experimental

1. Elevated Plus Maze (EPM)

Anxiolytic activity was measured using the elevated plus maze test. The maze consisted of two open (31 cm × 5 cm × 1 cm) and two closed (31 cm × 5 cm × 15 cm) arms, extending from a central platform (5 cm × 5 cm) and elevated to a height of 40 cm above the floor. Mice were individually placed on the center of the maze facing a closed arm, and the number of entries and the time spent in closed and open arms were recorded during a 6-min observation period. Arm entries were defined as entry of all four paws into an arm. The percentage time spent on open arms (100 × open/total time) was calculated for each animal.

2. Computerized analysis

The EPM was videotaped using the high-resolution video camera WV-CP244 (Panasonic, Secaucus, USA). The analysis of the videos was performed using TopScan, Top View Animal Behaviour Analyzing System (version 1.00, Clever Sys Inc. Preston, USA) by an unbiased and treatment-blinded person.

3. Animals

Male BL6/C57J mice between 6 and 12 weeks old and weighing 22–34 g were purchased from Harlan (Indianapolis, USA). Mice were housed in cages of 5 at 20 ± 1 °C in a 12-h light/dark cycle. Tap water and food pellets were available *ad libitum*. Groups of 10 mice were randomly assigned to different treatment groups and tested in a varying order. Animals were tested repeatedly under the same experimental conditions. All experiments were carried out in a quiet room under controlled light conditions between 8:00 a.m. and 1:00 p.m. All animals were housed and all experiments performed according to the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA.

4. Drugs

Diazepam (ampoules containing 10 mg/2 ml; Hoffmann-La Roche, Switzerland). Diazepam was diluted to 1.5 mg/10 ml with water (Millipore quality) containing 0.5% propylene glycol (Sigma-Aldrich). Three different concentrations (150 mg/kg, 375 mg/kg, and 600 mg/kg) of the PF extract (Merck Selbstmedikation GmbH, Germany) were prepared by dissolving the extracts in 10 ml deionized water with 0.5% propylene glycol to form a suspension. The extract is the sole active ingredient of the proprietary herbal drug Kytta-Sedativum® and a voucher specimen is deposited at the Department of Pharmaceutics, College of Pharmacy, University of Florida Gainesville, USA. (Voucher No.: #COP-PC-3). All solutions were prepared freshly on test days.

All animals were brought to the testing room at least 1 h prior to testing, and remained in the same room throughout the test. Animals were orally treated with control (vehicle), diazepam (1.5 mg/kg, n = 10), or the PF extract (corresponding to 150, 375 and 600 mg/kg, n = 10/each) 60 min before evaluation in the test models.

5. Statistics

Data were analyzed by two-way ANOVA and Dunnett's Test using Graphpad 4.0 Software, USA.

References

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