RESEARCH ARTICLE

Effect of myricetin on behavioral paradigms of anxiety

Mahalaxmi Mohan, Swati S. Jadhav, Veena S. Kasture, and Sanjay B. Kasture

Department of Pharmacology, MGV's College of Pharmacy, Nashik, Maharashtra, India

Abstract

Myricetin (10, 30, and 100 mg/kg) obtained from Vitis vinifera Linn. (Vitaceae) exhibited significant anxiolytic activity in behavioral models of anxiety, namely, elevated plus maze, light/dark apparatus, open field apparatus, and hole board apparatus, in mice. Myricetin significantly (p < 0.05) reduced lithium induced head twitches and also antagonized meta-chlorophenylpiperazine (m-CPP) induced anxiety, suggesting that it acts by modifying serotonin transmission. The cumulative concentration response curve (CCRC) of 5-HT in the presence of myricetin (10 µg/mL) was shifted toward the right in rat fundus.

Keywords: Anxiety; myricetin; serotonin; m-CPP; behavioral paradigms

Introduction

A large number of medicinal plants including Bacopa monniera L. Pennell. (Scrophulariaceae) (Bhattacharya & Ghosal, 1998), Albizia lebbeck L. Benth. (Mimosaceae) (Une et al., 2002), Sesbania grandiflora L. Poir. (Fabaceae) (Kasture et al., 2002), Korean ginseng Linn. (Araliaceae) (Mohan et al., 2005), Sausiaurea lappa C.B. Clarke (Asteraceae) (Ambavade et al., 2006), and Sphaeranthus indicus Linn. (Asteraceae) (Bodhankar et al., 2006) have been reported to possess anxiolytic activity. Vitis vinifera Linn. (Vitaceae), known as common grapevine or raisin, is widely cultivated throughout India. It has varied uses in the Ayurvedic and Unani systems of medicine. Raisins possess laxative, cooling, expectorant, antioxidant, diuretic, aphrodisiac, and stomachic properties (Warrier et al., 1995). Vitis vinifera grape seed also possesses adaptogenic and nootropic activities (Sreemantula et al., 2005). Vitis vinifera seeds contain flavonoids, mainly myricetin. Flavonoids are known to have anxiolytic activity (Ayoka et al., 2005; Mishra et al., 2007). Therefore, it was considered worthwhile to explore the anxiolytic potential using various behavioral paradigms such as the elevated plus maze (Lister, 1987), light/dark apparatus (Belzung et al., 1990), open field apparatus (Turner, 1972), and hole board apparatus (Clark et al., 1971). An attempt was made to postulate a mechanism for the anxiolytic study by the lithium induced head twitch (Wielosz & Kleinrok, 1979) model and meta-chlorophenylpiperazine (m-CPP) induced anxiety in the elevated plus maze.

Materials and methods

Preparation of the extract

Vitis vinifera raisins (1 kg), purchased locally, were authenticated by Dr. S. C. Pal, NDMVP Samaj’s College of Pharmacy, Nashik, India. A specimen sample of the same was preserved in the herbarium of the Botanical Survey of India, Pune, with voucher no. 14557830 for future reference. The cut pieces of raisins were defatted with petroleum ether (60–80°C) using a Soxhlet extractor, and the marc was successively extracted with methanol. The extract was concentrated under vacuum. The methanol extract was then hydrolyzed with 2N HCl for 30 min at 100°C. The cooled solution was extracted twice with ethyl acetate and the combined extracts were dried (yield 0.5% w/w) (Harborne, 1984). The presence of myricetin in the extract was confirmed by matching thin layer chromatography (TLC) of myricetin provided by Professor S. C. Pal (Department of Pharmacognosy, NDMVP Samaj’s College of Pharmacy, Nashik), using a solvent system comprising chloroform:water (1:1), with a retardation factor (Rf) value of 0.62 (Rusjan & Zora, 2007)
and by high-performance liquid chromatography (HPLC) using acetonitrile–pH 2.4 pyrophosphate buffer (1:3), the flow rate being 1.2 mL/min at 20°C, scanned at wavelength 266 nm. The retention time of myricetin was observed at 5.76 min (Tsuchiya, 1998; Tokusoglu et al., 2003). The myricetin content of the extract was 95%, w/w. Myricetin was dissolved in distilled water before being orally administered.

Animals
Albino mice (20–25 g) and albino rats (125–150 g) of both sexes aged 2–4 months were obtained from Serum Institute, Pune. Animals were housed in groups of five under standard laboratory conditions of temperature 25 ± 1°C with free access to food (Hindustan Lever, India) and water. Food but not water was withheld 4 h before the experiment. Experiments were performed during the light portion (09.00–14.00 h). The Institutional Animal Ethical Committee approved the protocol of this study.

Drugs
Lithium sulfate (HiMedia, Mumbai), m-CPP (Sigma-Aldrich, Mumbai), and serotonin (Sigma-Aldrich) were used for the study.

Anxiolytic study
Elevated plus maze test
The elevated plus maze (EPM) consisted of two open arms (25 × 5 cm) crossed with two closed arms (25 × 5 × 20 cm). The arms were connected by a central square of 5 × 5 cm. The maze was elevated to a height of 50 cm and placed inside a light and sound attenuated room (Lister, 1987). Groups of mice each containing five animals were treated with vehicle, diazepam (1 mg/kg, i.p.), and myricetin (10, 30, and 100 mg/kg, p.o.) 1 h before mice were placed individually in the EPM. The time spent in open arms, and entries into open and closed arms, were recorded for a period of 5 min. In another study, the effects of m-CPP (1 mg/kg, i.p.) on myricetin (10, 30, and 100 mg/kg, p.o.) were also tested. m-CPP was given 30 min prior to myricetin and the effect on the above parameters was noted.

Open field apparatus test
The apparatus consisted of a wooden box (96 × 96 × 50 cm). The floor of the box was divided into 16 squares (Turner, 1972). Groups of mice each containing five animals were treated with vehicle, diazepam (1 mg/kg, i.p.), and myricetin (10, 30, and 100 mg/kg, p.o.) 1 h before mice were placed individually in one corner of a square. Parameters such as number of rearings and number of squares crossed were recorded for 5 min.

Hole board apparatus test
The apparatus consisted of a wooden box (40 × 40 × 25 cm) with 16 holes (diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm (Clark et al., 1971). Groups of mice each containing five animals were treated with vehicle, diazepam (1 mg/kg, i.p.), and myricetin (10, 30, and 100 mg/kg, p.o.) 1 h before placing mice individually in the apparatus, and the number of head pokings were recorded for 5 min.

Light/dark apparatus test
Two equally sized boxes (20 × 20 × 14 cm, one dark and the other lit) were connected by a tunnel (5 × 7 × 10 cm). Mice in groups of five each were treated with vehicle, diazepam (1 mg/kg, i.p.), and myricetin (10, 30, and 100 mg/kg, p.o.) 1 h before placing mice individually in the lit area. The number of transitions and the time spent in the light box were recorded for 5 min (Belzung et al., 1990).

Lithium-induced head twitches (serotonin mediated behavior)
Rats were divided into four groups of five animals each. Rats received lithium sulfate (200 mg/kg, i.p.) 1 h after vehicle or myricetin (30 and 100 mg/kg, p.o.) treatment. The number of head twitches was recorded for 1 h after lithium sulfate administration (Wielosz & Kleinrok, 1979).

In vitro studies
Rat fundus was removed and placed in Krebs solution. Physiological salt solution had the following composition (mM): NaCl (118); KCl (4.7); CaCl₂ (2.5); MgSO₄ (1.2); NaHCO₃ (25); KH₂PO₄ (1.2); and glucose (11). The physiological salt solution had a pH of 7.4. It was warmed to 37°C and aerated with 95% O₂ and 5% CO₂ (Carbogen). One end was tied to an aerator tube and the other end to the frontal writing lever. Each strip was placed under optimum resting tension (1.5 g) and allowed to equilibrate for 30 min, with frequent changes of Krebs solution at 10 min intervals. The cumulative concentration response curve (CCRC) of serotonin (5-HT) in the presence and the absence of myricetin (10 µg/mL) was recorded for 90 s for each tissue preparation on a Sherrington recording drum (Goyal et al., 2000).

Statistics
All data are shown as mean ± SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnett’s test. Differences of $p < 0.05$ were considered statistically significant.
Results and discussion

Anxiolysis is mediated by a number of mechanisms in the central nervous system (CNS); an alternative explanation proposed for the anxiolytic effects of myricetin is via increased CNS secretion of β-endorphin (Liu et al., 2006). The elevated plus maze utilizes the premise that exposure of a rat or mouse to an elevated and open maze arm leads to an approach–avoidance conflict which is considerably stronger than that evoked by exposure to an enclosed arm. The vehicle treated control animals showed 31.77 ± 6.23 s, 4.28 ± 1.15, and 5 ± 0.75 as time spent in open arms, number of entries in open arms, and number of head dips, respectively. We observed that myricetin (10, 30, and 100 mg/kg) significantly (p<0.05) increased the time spent in open arms, number of entries in open arms, and number of head dips, as compared to controls (Table 1). An inverse-U dose–response relationship was observed, which is the characteristic of all anxiolytic drugs (Vishwakarma et al., 2002). The reversal of m-CPP (1 mg/kg, a 5-HT2B/2C agonist)-induced anxiety by myricetin indicates involvement of a serotonergic mechanism in the anxiolytic activity of myricetin (Table 2). In the light/dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behavior of rodents. Anxiolytics increase light to dark transitions and the time spent in the lit area. In our study, the vehicle treated control animals showed 52.8 ± 6.95 s and 5.6 ± 0.24 as time spent in the lit area and number of transitions. Myricetin (10, 30, and 100 mg/kg) increased the time spent in the lit area and myricetin (30 mg/kg) increased the number of transitions as compared to controls (Figure 1). In the open field test, the vehicle treated control animals showed 56 ± 5.89 and 15 ± 6.45 as the number of squares traversed and rearings, respectively. Myricetin (10, 30, and 100 mg/kg) resulted in a significant (p<0.05) increase in the number of squares traversed and an increased tendency to reach to the walls and rear, as compared to control animals. Rearings were significantly (p<0.05) increased by myricetin (30 and 100 mg/kg) (Figure 2). A decrease in locomotion is indicative of diminished dopaminergic transmission, which may be secondary to the rise in 5-HT level caused by anxiogenic agents (Jones et al., 1992; Kahn et al., 1988). Placing a mouse on the hole board apparatus, elevated to 25 cm from the table, induces anxiety as it is exposed to a new environment. In the hole board apparatus, the vehicle treated control animals showed 12.0 ± 2.16 as the number of head pokes. We observed a significant (p<0.05) increase in head poking with myricetin (10, 30, and 100 mg/kg) and diazepam (1 mg/kg) as compared to control animals (Figure 3). Lithium sulfate administered intraperitoneally to rats releases serotonin from the serotonergic neurons, which stimulates 5-HT, receptors to produce head twitches (Schreiber et al., 1995; Wielosz & Kleinrok, 1996).

Table 1. Effect of myricetin on time spent in open arms, entries in open and closed arms, and head dips in elevated plus maze.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment (mg/kg)</th>
<th>Time spent in open arm (s)</th>
<th>Entries in open arm</th>
<th>Entries in closed arm</th>
<th>Number of head dips</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>31.77 ± 6.23</td>
<td>4.28 ± 1.15</td>
<td>5 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diazepam (1)</td>
<td>76 ± 8.58*</td>
<td>8.8 ± 1.5</td>
<td>13.6 ± 1.93*</td>
<td>11.2 ± 2.35</td>
</tr>
<tr>
<td>3</td>
<td>Myricetin (10)</td>
<td>139.2 ± 5.52*</td>
<td>10.8 ± 0.37*</td>
<td>6.8 ± 1.2</td>
<td>14.2 ± 2.05*</td>
</tr>
<tr>
<td>4</td>
<td>Myricetin (30)</td>
<td>173.4 ± 2.87*</td>
<td>13.6 ± 1.91*</td>
<td>9.0 ± 1.7</td>
<td>14.0 ± 1.97*</td>
</tr>
<tr>
<td>5</td>
<td>Myricetin (100)</td>
<td>110.7 ± 18.11*</td>
<td>10.0 ± 1.86*</td>
<td>6.33 ± 0.71</td>
<td>10.17 ± 2.0</td>
</tr>
</tbody>
</table>

F4,10 = 5. The observations are mean ± SEM. *p<0.05, as compared to vehicle (ANOVA followed by Dunnett’s test).

Table 2. Effect of myricetin on m-CPP induced anxiety in elevated plus maze apparatus.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment (mg/kg)</th>
<th>Time spent in open arm (s)</th>
<th>Entries in open arm</th>
<th>Entries in closed arm</th>
<th>Number of head dips</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (m-CPP)</td>
<td>2.8 ± 0.33</td>
<td>0.4 ± 0.24</td>
<td>6.0 ± 1.14</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>Myricetin (10) + m-CPP (1)</td>
<td>2.4 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>2.2 ± 0.73*</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>3</td>
<td>Myricetin (30) + m-CPP (1)</td>
<td>13.2 ± 3.17</td>
<td>1.8 ± 0.86</td>
<td>2.2 ± 0.96*</td>
<td>1.2 ± 0.73</td>
</tr>
<tr>
<td>4</td>
<td>Myricetin (100) + m-CPP (1)</td>
<td>40.6 ± 7.35*</td>
<td>3.0 ± 0.83*</td>
<td>4.6 ± 1.03</td>
<td>2.0 ± 0.31*</td>
</tr>
</tbody>
</table>

F3,4 = 15.59 4.50 3.70 6.08

n=5. The observations are mean ± SEM. *p<0.05, as compared to vehicle (ANOVA followed by Dunnett’s test).

Figure 1. Effect of myricetin on time spent in lit zone and number of transitions in light/dark box apparatus. Group 1 = control, group 2 = diazepam (1), group 3 = myricetin (10), group 4 = myricetin (30), group 5 = myricetin (100) (n=5). The observations are mean ± SEM. *p<0.05, as compared to vehicle (ANOVA followed by Dunnett’s test).
Drugs that block 5-HT$_2$ receptors antagonize the head twitches. The observed reduction in number of head twitches may be due to direct and indirect actions of myricetin (30 and 100 mg/kg) on the 5-HT system. Thus, lithium-induced head twitches were significantly and dose-dependently reduced in myricetin treated rats (Figure 4). 5-HT$_2$ antagonistic activity of myricetin was also depicted by a shift of the CCRC of 5-HT toward the right, with suppression of the maxima, in the rat fundus (Figure 5). Thus, it is concluded that myricetin exhibits anxiolytic activity by modifying 5-HT transmission.

**Declaration of interest:** The authors alone are responsible for the content of this paper.
Anti-anxiety effects of myricetin


