Neuropharmacological Actions of *Pluchea indica* LESS Root Extract in Socially Isolated Mice

Suchitra THONGPRADITCHOTE,^{a,1)} Kinzo MATSUMOTO,^a Rungravi TEMSIRIRIRKKUL,^b Michihisa TOHDA,^a Yukihisa MURAKAMI,^a and Hiroshi WATANABE^{*,a}

Division of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University,^a 2630 Sugitani, Toyama-shi, Toyama 930-01, Japan, and Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University,^b Bangkok 10400, Thailand.

Received September 11, 1995; accepted November 13, 1995

The effects of *Pluchea indica* LESS root extract (PI-E) on locomotor activity and pentobarbital-induced sleep, social isolation-induced aggressive behavior, motor coordination in the rotarod test, pentylenetetrazole-induced convulsion and nociceptive responses in the tail-pinch test were examined in mice. Socially isolated mice showed higher locomotor activity and shorter duration of pentobarbital sleep than group-housed mice. PI-E (50–100 mg/kg, *p.o.*) significantly decreased locomotor activity and prolonged pentobarbital sleep in a dose-dependent manner in isolated mice but not in group-housed mice. At a large dose (400 mg/kg, *p.o.*), PI-E not only decreased locomotor activity but also prolonged pentobarbital sleep in group-housed mice. The reference drug diazepam, at 0.5 mg/kg, also suppressed the locomotor activity in isolated mice but not in group-housed mice. Moreover, diazepam, at 0.1 and 0.5 mg/kg, significantly prolonged pentobarbital sleep in both isolated mice and group-housed mice. The effects of PI-E and diazepam on pentobarbital sleep in isolated mice were significantly attenuated by flumazenil (1 mg/kg, *i.v.*). PI-E (50–100 mg/kg), as well as diazepam (0.5–5 mg/kg, *p.o.*), dose-dependently suppressed social isolation-induced aggressive behavior, but it had no effect on pentylenetetrazole-induced convulsion, motor coordination in the rotarod test, or nociceptive response in the tail pinch test in group-housed mice. These results suggest that PI-E attenuates pathophysiological changes caused by social isolation stress in mice, and that the GABAergic system is partly involved in the action of PI-E on a social isolation-induced decrease in pentobarbital sleep.

Key words *Pluchea indica*; locomotor activity; aggressive behavior; pentobarbital sleep; socially isolated mouse; benzodiazepine receptor

Pluchea indica LESS is a shrub of the family Compositae and is widespread in southeast Asia. In this area, various parts of *Pluchea indica* LESS have been used as a folk medicine for the treatment of dysuria, hemorrhoids and disorders causing cachexia or wasting.²⁾ In laboratory animals, the plant appears to produce diuretic activity without acute toxicity.^{2,3)} Moreover, many studies have demonstrated the anti-ulcer and anti-inflammatory effects of the *Pluchea indica* LESS root extract (PI-E) in rodents.^{4–7)} Recently, Sen and Nag Chaudhuri⁸⁾ showed that the systemic administration of PI-E reduced spontaneous motor activity, caused hypothermia, prolonged pentobarbital-induced sleeping time, and inhibited the electroshock-induced fighting in mice. They also found that the extract caused an increase in the γ -aminobutyric acid (GABA) level in mouse brain. In the present study, to further clarify the pharmacological profiles of PI-E, and possible mechanisms of action of PI-E, we investigated the effects of this plant extract on spontaneous motor activity, muscle motor coordination in a rotarod test, pentobarbital-induced sleeping time, nociceptive response in the tail pinch test, pentylenetetrazole-induced convulsion, and social isolation-induced aggressive behavior in mice.

MATERIALS AND METHODS

Materials Authentication of *P. indica* LESS was achieved by comparison with herbarium specimens in the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand.

Preparation of PI-E The dried powdered root of *Pluchea indica* LESS was extracted with petroleum-ether using a Soxhlet apparatus. After the petroleum-ether fraction was separated, the residue was again extracted with chloroform. The chloroform fraction was removed and the residue was extracted with methanol. The methanol fraction was evaporated under reduced pressure to yield a deep brown resinous mass that was used as PI-E. The yield of the extract was 6.6% w/w in terms of the starting material.

Animal Male 5-week-old ddY mice (Japan SLC, Shizuoka, Japan) were used in the experiments. Animals were housed in groups of 20–25 per cage (35 × 30 × 16 cm) for at least one week in the laboratory animal room before the experiments, with free access to food and water. In some experiments, 4-week-old ddY mice were either housed in groups of five (group-housed mice) or housed individually (isolated mice) in a Plexiglas cage (24 × 17 × 12 cm) for 6–7 weeks before the experiments as previously reported.^{9,10)} Housing conditions were thermostatically maintained temperature at 23 ± 1 °C with 60% humidity and a 12 h light–dark cycle (light on 7:30–19:30).

Spontaneous Locomotor Activity Mice were transferred to the laboratory from the animal room at least 2 h before starting the experiments. The vehicle, PI-E or the reference drug, diazepam, was orally (*p.o.*) administered and the mouse was placed individually in a small cage (24 × 17 × 12 cm). After 30 min, each mouse was placed individually in a Plexiglas cage (25 × 18 × 24 cm) fixed at the center of the Scanet SV-10 system (Toyo Sangyo Co., Ltd., Toyama, Japan) as previously described,¹¹⁾ and then

* To whom correspondence should be addressed.

changes in locomotor activity were recorded over a 30-min period.

Pentobarbital-Induced Sleep Vehicle, PI-E, or diazepam was administered *p.o.* to group-housed or socially isolated mice. When testing the antagonistic effect of flumazenil, flumazenil (1 mg/kg) was injected *i.v.* immediately before administration of the test drugs. After 30 min, pentobarbital (50 mg/kg) was injected *i.p.* and the duration of pentobarbital-induced sleep was measured as the period between the loss of the righting reflex and its return.^{12,13)}

Aggressive Behavior in Socially Isolated Mice When testing the aggressive behavior, PI-E, diazepam or the vehicle was administered *p.o.* 30 min before starting the experiments. Measurement of the aggressive responses was performed as previously reported.^{9,10,13)} Briefly, two isolated mice were placed in a neutral cage (24 × 17 × 12 cm) of the same size as their home cages, and the total duration of biting attacks, wrestling, or both observed during a 20-min period was measured. The latency period before the first attack was also recorded using a keyboard interfaced with a computer (PC 9801NS, NEC, Tokyo, Japan).

Rotarod Test Effect of PI-E on motor coordination was examined in the rotarod test by modifying the method of Dunham and Miya.¹⁴⁾ Briefly, mice were placed on a rotating rod (24 mm diameter, rotating at 6 rpm) in a pre-test session, and only the animals that stayed on the rod for 3 min and over were selected from the pre-test session and used for the test session. The test session was performed on the same day as the pre-test. In the test session, ten mice were used for each drug treatment. Thirty min after the administration of test drugs, mice were placed on the rod. If the animal failed to remain on the rod no more than 3 min, the test was judged to be positive and the number of animals that showed a positive reaction was counted.

Pentylenetetrazole-Induced Convulsion PI-E (100, 200 and 400 mg/kg), diazepam (10 mg/kg) or vehicle was *p.o.* administered. After 30 min, pentylenetetrazole (100 mg/kg) was injected *i.p.*, and the number of animals that died during a 30-min observation period was recorded.¹⁵⁾

Antinociceptive Activities in the Tail Pinch Test The nociceptive response in the tail pinch test was measured according to Haffner's method, as previously reported.¹⁶⁾ Briefly, mice were pretested by pinching their tails with a crammer (500 g pressure), and only the animals which showed nociceptive responses, such as biting the crammer within 2 s, were used for the experiments. Thirty min after *p.o.* administration of the test drugs, the latency of nociceptive responses was measured.

Drugs The drugs used were as follows: pentobarbital sodium (Tokyo Kasei Kogyo, Tokyo, Japan), aspirin (Iwaki Seiyaku, Tokyo, Japan), morphine sulfate (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), diazepam (Cercine® Inj.; Takeda Chemical Industries, Ltd., Osaka, Japan), Flumazenil® (Roche Co., Ltd., Basel), acetic acid and carboxymethyl cellulose sodium salt (Nacalai Tesque, Inc., Kyoto, Japan). The *P. indica* root extract, diazepam and aspirin were suspended in 0.5% carboxymethylcellulose sodium (CMC) solution. The other chemicals were dissolved in physiological saline. All

drug solutions were prepared immediately before starting the experiments and were administered in a constant volume of 10 ml/kg body weight.

Statistical Analysis The effect of each drug on aggressive behavior was analyzed using the Kruskal-Wallis test followed by the Mann-Whitney *U*-test. Effects on locomotor activity, pentobarbital-induced sleep and nociceptive responses were evaluated by a one-way ANOVA followed by Dunnett's test, or a two-way ANOVA followed by the Student-Newman-Keuls test. The difference was considered statistically significant at $p < 0.05$.

RESULTS

Systemic administration of PI-E, at doses up to 5 g/kg, did not produce any mortality in mice. Neither loss of righting reflex nor ataxia was observed following the administration of 5 g/kg PI-E (*p.o.*).

Effect on Locomotor Activity in Group-Housed and Socially-Isolated Mice Consistent with our previous data,^{9,10)} socially isolated mice showed significantly higher locomotor activity than group-housed mice [Fig. 1; $F_{\text{housing condition}}(1, 62) = 41.676$, $p < 0.001$]. PI-E, at 50 and 100 mg/kg, had no effect on the activity of group-housed control mice, but it significantly decreased that of socially isolated mice in a dose-dependent manner [$F_{\text{PI-E}}(2, 48) = 3.555$, $p < 0.05$]. The reference drug diazepam (0.5 mg/kg) also significantly suppressed the spontaneous locomotor activity in isolated mice without affecting the activity in group-housed control mice [$F_{\text{diazepam}}(1, 32) = 5.873$, $p < 0.05$]. On the other hand, the extract, at 400 mg/kg, significantly decreased locomotor activity in group-housed mice (Fig. 2).

Effect on Pentobarbital-Induced Sleep in Group-Housed and Socially Isolated Mice Socially isolated mice exhibited a significantly shorter duration of pentobarbital

Fig. 1. Effect of Smaller Doses of PI-E on Spontaneous Motor Activity in Isolated and Group-Housed Mice

CMC (0.5%), PI-E or the reference drug diazepam was orally administered. After 30 min, each mouse was placed individually in a small cage (24 × 17 × 12 cm) fixed at the center of the Scanet SV-10 and changes in locomotor activity were recorded over a 30-min period. Each datum represents the mean ± S.E.M. of 10 animals. # $p < 0.01$ compared with vehicle-treated group-housed mice. * $p < 0.05$ compared with vehicle-treated isolated group (Student-Newman-Keuls test).

Fig. 2. Effect of Larger Doses of PI-E on Spontaneous Motor Activity in Group-Housed Mice

After administration of test agents, spontaneous motor activity was measured as described in Fig. 1. Each datum represents the mean $\pm$ S.E.M. of 10 animals. * $p < 0.05$ compared with vehicle control (Student-Newman-Keuls test).

Fig. 3. Effect of Smaller Doses of PI-E on Pentobarbital-Induced Sleep in Group-Housed and Socially Isolated Mice

Thirty min after *p.o.* administration of 0.5% CMC, PI-E or diazepam, pentobarbital (50 mg/kg, *i.p.*) was injected and the duration of pentobarbital-induced sleep was measured as the period between the loss of the righting reflex and its return. Each datum represents the mean $\pm$ S.E.M. of 8 mice. # $p < 0.05$, ** $p < 0.01$ compared with vehicle-treated group-housed mice. * $p < 0.05$ compared with vehicle-treated isolated group (Student-Newman-Keuls test).

(50 mg/kg)-induced sleep than the group-housed control mice [$F_{\text{housing condition}}(1, 56) = 36.768$, $p < 0.001$]. As shown in Fig. 3, PI-E, at smaller doses (50 and 100 mg/kg), dose-dependently reversed the hypnotic activity of pentobarbital decreased by social isolation to the level of group-housed control mice, but the same doses of PI-E did not affect the pentobarbital sleep in the group-housed mice [$F_{\text{housing condition} \times \text{PI-E}}(2, 42) = 12.818$, $p < 0.001$]. On the other hand, PI-E at larger doses (200 and 400 mg/kg) significantly prolonged pentobarbital sleep in group-housed mice in a dose-dependent manner (Fig. 4). The reference drug diazepam (0.1–0.5 mg/kg) significantly prolonged pentobarbital sleep in both group-housed and socially isolated mice [Figs. 3 and 4; $F_{\text{housing condition} \times \text{diazepam}}(1, 28) = 42.475$, $p < 0.001$ in Fig.

Fig. 4. Effect of Larger Doses of PI-E on Pentobarbital-Induced Sleep in Group-Housed Mice

Thirty min after *p.o.* administration of test agents, pentobarbital (50 mg/kg, *i.p.*)-induced sleep was measured as described in Fig. 3. Each datum represents the mean $\pm$ S.E.M. of 10 animals. ** $p < 0.01$ compared with vehicle control group (Dunnett's test).

3].

Effect of Flumazenil and Diazepam-Induced Modulation of Pentobarbital Sleep in Socially Isolated Mice A two-way ANOVA revealed a significant PI-E (100 mg/kg) $\times$ flumazenil (1 mg/kg) interaction in terms of pentobarbital sleep in socially isolated mice [$F(1, 36) = 23.736$; $p < 0.001$]. Moreover, a post hoc test indicated that 1 mg/kg flumazenil significantly attenuated the effect of PI-E on pentobarbital sleep in isolated mice without affecting the sleep by itself (Fig. 5A). In group-housed mice, no significant flumazenil $\times$ 200 mg/kg PI-E interaction was observed [$F(1, 36) = 0.087$, $p > 0.05$; Fig. 5B]. On the other hand, a significant diazepam $\times$ flumazenil interaction was observed in terms of pentobarbital sleep in socially isolated mice [$F(1, 36) = 24.834$; $p < 0.001$]. Flumazenil completely abolished the prolonging effect of 0.1 mg/kg diazepam on pentobarbital sleep in isolated mice (Fig. 5C).

Effect on Aggressive Behavior in Socially Isolated Mice PI-E (50 and 100 mg/kg) and the reference drug diazepam (0.5–5 mg/kg) dose-dependently decreased the duration of aggressive behavior in socially isolated mice (Fig. 6). The decrease in the duration of aggressive behavior by the extract was accompanied by a significant increase in the latency before showing aggressive behavior. The reference drug diazepam (0.5–5 mg/kg) also dose-dependently decreased the duration of aggressive behavior and showed a tendency to prolong the latency of aggressive behavior.

Effect on Pentylentetrazol-Induced Convulsion PI-E (100–400 mg/kg) had no effect on pentylentetrazole-induced convulsion, whereas the reference drug diazepam (10 mg/kg) completely blocked the effect of pentylentetrazole (data not shown).

Effect on Motor Activity in the Rotarod Test The reference drug diazepam (1, 5 and 10 mg/kg) dose-dependently exhibited muscle relaxant activity in the rotarod test, whereas PI-E, at 400 and 800 mg/kg, failed

Fig. 6. Effects of PI-E and Diazepam on Aggressive Behavior in Socially Isolated Mice

PI-E and diazepam were administered *p.o.* 30 min before the experiments. Each datum represents the mean $\pm$ S.E.M. of 7–8 pairs of mice. * $p < 0.05$; ** $p < 0.01$ compared with vehicle control (Mann Whitney *U*-test).

to produce muscle relaxant action in group-housed normal mice (Table 1).

Effect on Nociceptive Responses in the Tail Pinch Test In the tail pinch test, the reference drug morphine, but not PI-E (100–400 mg/kg), significantly prolonged the latency in showing nociceptive responses (Table 2).

DISCUSSION

Sen and Nag Chaudhuri⁸⁾ have reported that an i.p.

Fig. 5. Antagonistic Effect of Flumazenil on PI-E-Induced Modulation of Pentobarbital Sleep in Socially Isolated and Group-Housed Mice

The PI-E (A, B), the reference drug diazepam (C) or the vehicle was administered *p.o.* to socially isolated mice. Immediately before the administration of test agents, flumazenil (1 mg/kg, i.v.) was injected. After 30 min, pentobarbital (50 mg/kg, i.p.) was injected. Each datum represents the mean $\pm$ S.E.M. of 10 animals. * $p < 0.05$ compared with vehicle alone. # $p < 0.05$ compared with PI-E or diazepam alone (Student-Newman-Keuls test).

Table 1. Effects of *P. indica* Root Extract and Diazepam on Muscle Relaxant Activity

Drug	Dose (mg/kg, <i>p.o.</i>)	% of animals showing muscle relaxation
0.5% CMC	—	0
<i>P. indica</i> root extract	400	0
	800	0
Diazepam	1	10
	5	50
	10	100

Thirty min after administration of test drugs, mice were placed on the rod and the number of animals that showed muscle relaxation was recorded. The total number of animals used in each drug treatment was 10. Each point represents the mean $\pm$ S.E.M. of 10 animals.

Table 2. Effects of *P. indica* Root Extract and Morphine on the Nociceptive Responses in the Tail Pinch Test

Drug	Dose (mg/kg, <i>p.o.</i>)	Response time (s)
0.5% CMC	—	0.98 $\pm$ 0.05
<i>P. indica</i> root extract	100	1.00 $\pm$ 0.05
	200	1.18 $\pm$ 0.11
	400	1.21 $\pm$ 0.12
Morphine	5	1.21 $\pm$ 0.12
	10	2.30 $\pm$ 0.13**

Each value shows the mean $\pm$ S.E.M. of 10 mice. ** $p < 0.01$ compared with vehicle control group (Dunnett's test).

injection of PI-E produces a reduction in spontaneous motor activity, prolongation of pentobarbital sleep and suppression of electroshock-induced aggressive behavior in mice. Consistent with their findings, PI-E attenuated spontaneous locomotor activity at 400 mg/kg (*p.o.*) and prolonged pentobarbital-induced sleep at ≥ 200 mg/kg (*p.o.*) in group-housed mice. In this study, we found that PI-E exhibited neuropharmacological actions in socially isolated mice at smaller doses which had produced no

effect in the group-housed mice, and that these action profiles of *P. indica* were partly similar to those of diazepam.

Long-term social isolation is known to cause various behavioral and neurochemical changes in mice,¹⁷⁻¹⁹⁾ and it has been used as a model to elucidate drug action on aggression and/or social behavioral deficits.^{18,20,21)} In our previous reports, we have demonstrated that socially isolated mice exhibit higher spontaneous locomotor activity and shorter duration of pentobarbital sleep than group-housed mice.^{9,10,13)} Thus, the present data suggest that PI-E is more effective in the pathophysiological state caused by social isolation than in the normal state.

In this study, 1 mg/kg flumazenil, a selective benzodiazepine receptor antagonist, significantly attenuated the effects of 100 mg/kg PI-E and 0.1 mg/kg diazepam on pentobarbital sleep in isolated mice, whereas it failed to block the prolonging effect of 200 mg/kg PI-E on sleep in group-housed mice. Recent experiments in this laboratory have shown that functional changes in noradrenergic, corticotropin-releasing factor (CRF) and GABAergic systems in the brain are involved in the social isolation-induced decrease in the hypnotic activity of pentobarbital¹³⁾ (Matsumoto *et al.*, unpublished data). Therefore, it is possible that the GABA-benzodiazepine receptor system is partly implicated in the mechanism of action of this plant extract on pentobarbital-induced sleep in isolated mice.

The PI-E has been reported to increase GABA concentration in normal mouse brain.⁸⁾ However, taking into account the fact that the prolonging effect of 200 mg/kg PI-E on pentobarbital sleep in group-housed mice was not suppressed by 1 mg/kg flumazenil, the contribution of the GABAergic system to the action of large doses of PI-E may be small, if any, in normal animals. Moreover, it is possible that the effects of large doses of PI-E on locomotor activity and pentobarbital sleep in group-housed mice represent neurotoxicity or a neurological deficit rather than the pharmacological actions of this plant extract. However, such a possibility seems to be excluded for several reasons. Firstly, PI-E did not show any mortality, even at doses up to 5 g/kg, when it was orally administered. Secondly, PI-E at 400 and 800 mg/kg did not disturb motor coordination in the rotarod test or affect nociceptive responses in the tail pinch test.

PI-E attenuated social isolation-induced aggressive behavior at the same dose range as it suppressed the spontaneous locomotor activity of isolated mice. This

anti-aggressive effect of PI-E is consistent with the finding by Sen and Nag Chaudhuri⁸⁾ that PI-E also suppressed electroshock-induced aggressive behavior in mice. Taken into account that PI-E failed to affect motor coordination in the rotarod test, PI-E may have an anxiolytic-like psychoactivity.

In conclusion, the present results suggest that PI-E attenuates pathophysiological changes caused by social isolation in mice, and that the GABAergic system is partly involved in the action of PI-E on the social isolation-induced decrease in pentobarbital sleep. Clarification of the exact mechanisms of the action of PI-E will require further investigations of the active principle(s) responsible for its psychoactivity.

REFERENCES AND NOTES

- 1) S. T. is the recipient of a scholarship from the Ministry of Education, Culture and Sciences, Japan.
- 2) Farnsworth N. R., Bunyaphatsara N. (ed.), "Thai Medicinal Plants," Prachachon Co., Ltd., Bangkok, 1992, pp. 200-201.
- 3) Nilvise N., Vamnatjinda V., Vanveerakul B., Pidech P., *Thai J. Pharmacol.*, **11**, 1 (1989).
- 4) Pal S., Nag Chaudhuri A. K., *Phytother. Res.*, **3**, 156 (1989).
- 5) Sen T., Nag Chaudhuri A. K., *Planta Med.*, **56**, 661 (1990).
- 6) Sen T., Nag Chaudhuri A. K., *J. Ethnopharmacol.*, **33**, 135 (1991).
- 7) Sen T., Ghosh T. K., Nag Chaudhuri A. K., *Life Sci.*, **52**, 737 (1992).
- 8) Sen T., Nag Chaudhuri A. K., *Phytother. Res.*, **6**, 175 (1992).
- 9) Matsumoto K., Cai B., Satoh T., Ohta H., Watanabe H., *Pharmacol. Biochem. Behav.*, **39**, 167 (1991).
- 10) Cai B., Matsumoto K., Ohta H., Watanabe H., *Pharmacol. Biochem. Behav.*, **44**, 519 (1993).
- 11) Asakura W., Matsumoto K., Ohta H., Watanabe H., *Pharmacol. Biochem. Behav.*, **48**, 43 (1994).
- 12) Ferrini R., Miragoli G., Taccardi B., *Arzneim-Forsch.*, **24**, 2029 (1974).
- 13) Ojima K., Matsumoto K., Tohda M., Watanabe H., *Brain Res.*, **684**, 87 (1995).
- 14) Dunham N. W., Miya T. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 208 (1957).
- 15) Soaje-Echaque E., Lim R. K. S., *J. Pharmacol. Exp. Ther.*, **138**, 224 (1962).
- 16) Nguyen T. T. H., Matsumoto K., Nguyen M. D., Yamasaki K., Nguyen T. N., Watanabe H., *Pharmacol. Biochem. Behav.*, **52**, 427 (1995).
- 17) Modigh K., *Psychopharmacologia*, **33**, 1 (1973).
- 18) Rolinski Z., *Pol. J. Pharmacol.*, **27**, 37 (1975).
- 19) Tizabi Y., Massari V. J., Jacobowitz D. M., *Brain Res. Bull.*, **5**, 81 (1980).
- 20) Frances H., Lienard C., *Pharmacol. Biochem. Behav.*, **34**, 293 (1989).
- 21) Sofia R. D., *Life Sci.*, **8**, 705 (1969).