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Effects of Chinese and Paraguayan Medicinal Plants on the Duration of Immobility of Mice in the Forced Swimming Test

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Extracts of 5 Chinese and 3 Paraguayan medicinal plants were examined for their effects on the duration of the forced swimming-induced immobility in mice to evaluate their antidepressant activities. In the case of MeOH extracts, *Ginseng Radix* was the most active followed by *Rosmarinus officinalis* LINN. In the case of 30% EtOH extracts, the activities were in the decreasing order, *Psidium guajava* LINN., *Rosmarinus officinalis* LINN., *Valerianae Radix* and *Zizyphi Fructus*. Hot 30% EtOH extracts of *Zizyphi Fructus* and *Theobroma cacao* L. showed potent activities. The activities of EtOAc solubles of *Psidium guajava* LINN. and H_2O solubles of *Valerianae Radix* at 100 mg/kg were comparable to that of imipramine at 20 mg/kg.

Keywords——Chinese crude drug; Paraguayan crude drug; forced swimming test; duration of immobility; antidepressant activity

In our screening program for the pharmacologically active substances from medicinal plants, we found that many crude drugs and natural substances possess an antidepressant activity. Recently, we isolated antidepressant compounds by activity-directed fractionation of MeOH extracts of *Valeriana fauriei* and *Lobelia inflata* L.[1-3] It has been reported that the forced swimming test using mice or rats is selectively sensitive to clinically effective antidepressant drugs[4] and non-pharmacological therapeutic techniques such as electroconvulsive shock[5,6] and rapid eye movement (REM) sleep deprivation.[7,8] It is well known that the efficacy of clinically effective antidepressant drugs such as imipramine and mianserine in the test is closely related to the clinical data.[9-11] These facts, therefore, suggested that some plant extracts examined in this experiment might be potential clinically beneficial antidepressant crude drugs. Furthermore, the detailed studies of active constituents in these plants are of great value for finding a new type of antidepressant crude drugs. In this paper, we report the pharmacological effects of *Valerianae Radix* used as a sedative and an antispasmodic drug and 6 Chinese and Paraguayan medicinal plants known to have a sedative activity on the duration of immobility in mice in the forced swimming test.[12]

MATERIALS AND METHODS

Materials Chinese medicinal plants (*Valerianae Radix, Ginseng Radix* and *Zizyphi Fructus*) were obtained from Matsuura Yakugyo Co., Ltd. and Paraguayan medicinal plants (*Psidium guajava* LINN., *Rosmarinus officinalis* LINN. and *Astroeupatorium inulaefolium* K. et R.) were obtained from NKK Corporation.

Methods

Preparation of extracts Method A—1 kg of each material was immersed in MeOH (5 *l*) at room temperature for 24 h and MeOH was evaporated under reduced pressure to give MeOH extracts (yield of extracts; *Valerianae Radix*: 124 g, *Ginseng Radix*: 90 g, *Zizyphi Fructus*: 107 g).

Method B—1 kg of each material was immersed in aq EtOH (3 : 7=EtOH : H_2O v/v) (5 *l*) at room temperature for 24 h and the solvent was evaporated under reduced pressure to give 30% EtOH extracts (yield of extracts; *Valerianae Radix*: 58 g, *Zizyphi Fructus*: 42 g, *Psidium guajava*: 60 g, *Rosmarinus officinalis*: 39 g, *Astroeupatorium inulaefolium*: 37 g).

Method C—Plants (2 kg) were extracted with aq EtOH (3 : 7=EtOH : H_2O v/v) at 70℃ for 2 h. The extracts were separated by a centrifugal dehydrator at 60℃ followed by concentration under reduced pressure at 55℃. The concentrated materials were centrifuged to give hot 30% EtOH extracts (yield of extracts; *Valerianae Radix*: 1 kg, *Zizyphi Fructus*: 1 kg, *Theobroma cacao* L.: 450 g, *Triticum aestivum*: 800 g).

Method D—Husks of *Theobroma cacao* L. (2 kg) were smashed into about 2 mm pieces and roasted at 160℃ for 1 h in an electric oven. To this material, aq EtOH (95 :

5=EtOH : H_2O v/v) was added and heated to reflux temperature. After 1 h, the solvent was removed under reduced pressure and the residue was filtered to give a cacao extract (45 g).

Pharmacological test Male ddY mice (Seimi Co., Ltd.) weighting 24-27 g were used. They were kept under the standard laboratory conditions (room temperature 23±1℃, lighting cycle of 12 h light and 12 h darkness). The samples were dispersed in physiological saline containing Tween 80 (0.5% w/v) at concentrations of 1 mg/100 μl. The duration of immobility of mouse was measured by using the modified Porsolt method.[12] Briefly, mice were individually placed for 5 min in a vertical glass cylinder (20 cm height; 10 cm diameter), containing water (25-26℃) of depth 8 cm. Then, the mice were removed from the cylinder and allowed to dry themselves in a drying room. On the following day, 1 h after intraperitoneal injection of the samples, they were again put into the glass cylinder with water and the total duration of immobility was measured during the following 5 min period. The mice were judged immobile if they assumed floating posture in the water in a slightly hunched but upright position with their heads above the water surface. The test was performed during daytime. Six to 10 mice were used for each assay.

Statistics The results were assessed by Student's *t*-test.

RESULTS AND DISCUSSION

Effects of crude extracts of medicinal plants

The effects of MeOH extracts of the crude drugs (prepared by the method A) on immobility of mice in the forced swimming test are summarized in TABLE I. Of the 6 extracts, *Ginseng Radix* extract (at 100 mg/kg) the most potent of the test samples, was demonstrated to be comparable to that of imipramine (at 20 mg/kg), whereas *Rosmarinus officinalis* LINN. extract markedly decreased the duration of immobility. Other extracts apparently had no effects on the duration of immobility. The effects of 30% EtOH extracts (prepared by the method B) are summarized in TABLE II. Extracts of *Valerianae Radix, Zizyphi Fructus, Psidium guajava* LINN. and *Rosmarinus officinalis* LINN. decreased the duration of immobility in the test. Of these 4 extracts, the 30% EtOH extract of *Psidium guajava* LINN. showed a fairly potent activity. TABLE III lists the effects of hot 30% EtOH extracts (prepared by the method C) and of the cacao extract (prepared by the method D). Both *Zizyphi Fructus* and *Theobroma cacao* L. extracts had slightly decreased the duration of immobility, whereas neither *Valerianae Radix* nor *Triticum aestivum* L. produced any remarkable change in it. As shown in TABLES I, II and III, the MeOH extract of *Ginseng Radix* prepared by the method A was very active. The 30% EtOH extract of *Psidium guajava* LINN. prepared by the method B showed potent activity, whereas that of *Zizyphi Fructus* prepared by the methods B and C produced moderate decrease in the duration of immobility. The

TABLE I. Effects of MeOH Extracts of Medicinal Plants and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice

Plants	Sample administered (mg/kg)	Relative duration of immobility (%)[a]
Control[b]	—	100.0±17.5
Valerianae Radix	100	119.4±11.8
Ginseng Radix	100	42.4±17.8*
Zizyphi Fructus	100	157.0±45.3
Psidium guajava LINN.	100	174.0±16.4
Rosmarinus officinalis LINN.	100	77.6±30.1
Astroeupatorium inulaefolium K. et R.	100	104.7±20.4
Imipramine	20	51.0±9.5*

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly different from the control *$p<0.05$.

TABLE II. Effects of 30% EtOH Extracts of Medicinal Plants and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice

Plants	Sample administered (mg/kg)	Relative duration of immobility (%)[a]
Control[b]	—	100.0±8.3
Valerianae Radix	100	88.9±30.7
Zizyphi Fructus	100	93.9±31.7
Psidium guajava LINN.	100	66.3±11.5*
Rosmarinus officinalis LINN.	100	86.2±24.3
Astroeupatorium inulaefolium K. et R.	100	127.9±35.7
Imipramine	20	51.0±9.5*

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly defferent from the control *$p<0.05$.

TABLE III. Effects of Hot 30% EtOH Extracts of Medicinal Plants and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice

Plants	Sample administered (mg/kg)	Relative duration of immobility (%)[a]
Control[b]	—	100.0±15.5
Valerianae Radix	300	128.7±22.4
Zizyphi Fructus	300	83.0±12.9
Theobroma cacao L.	300	83.7±13.7
Triticum aestivum L.	300	129.8±18.5
Imipramine	20	51.0±9.5*

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly defferent from the control *$p<0.05$.

TABLE IV. Effects of Partitioned Fractions of MeOH Extracts and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice

Plants	Sample administered (mg/kg)	Relative duration of immobility (%)[a]		
		Partitioned fractions		
		EtOAc	BuOH	H_2O
Control[b]	—	100.0±12.3	100.0±19.4	100.0±10.7
Valerianae Radix	100	90.3±17.0	109.4±20.4	
Ginseng Radix	100	129.6±23.2	72.0±27.2	52.1±15.7*
Zizyphi Fructus	100	141.0±36.2	179.8±32.7	
Psidium guajava LINN.	100	51.3±16.1*	74.6±36.2	46.1±11.5*
Rosmarinus officinalis LINN.	100	106.0±23.8	13.2±10.0*	
Astroeupatorium inulaefolium K. et R.	100	89.6±24.1		
Imipramine	20	51.0±9.5*		

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly defferent from the control *$p<0.05$.

TABLE V. Effects of Partitioned Fractions of 30% EtOH Extracts and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice

Plants	Sample administered (mg/kg)	Relative duration of immobility (%)[a]		
		Partitioned fractions		
		EtOAc	BuOH	H_2O
Control[b]	—	100.0±9.5	100.0±14.4	100.0±3.8
Valerianae Radix	100	125.3±11.8	68.2±29.4	95.5±20.1
Zizyphi Fructus	100	82.6±32.0	103.5±26.9	
Psidium guajava LINN.	33		79.6±22.6	101.7±18.7
Rosmarinus officinalis LINN.	100	167.2±41.7	194.2±18.1	218.1±18.0
Astroeupatorium inulaefolium K. et R.	100	130.6±26.4	92.8±35.0	193.9±28.8
Imipramine	20	51.0±9.5*		

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly defferent from the control *$p<0.05$.

TABLE VI. Effects of Partitioned Fractions of Cacao Extracts and Imipramine on the Duration of Immobility in the Forced Swimming Test in Mice.

Plant	Sample administered (mg/kg)	Relative duration of immobility (%)[a]		
		Partitioned fractions		
		EtOAc	BuOH	H_2O
Control[b]	—	100.0±6.0	100.0±11.6	100.0±9.8
Theobroma cacao L.	300	63.9±9.8*	103.8±10.2	83.8±21.6
Imipramine	20	51.0±9.5*		

[a] Each value represents the mean±S.E.
[b] Physiological saline containing Tween 80 (0.5% w/v).
Significantly defferent from the control *$p<0.05$.

extracts of *Rosmarinus officinalis* LINN. prepared by both the methods A and B slightly decreased the duration of immobility. The extract of *Astroeupatorium inulaefolium* K. et R. prepared by both the methods A and B had no effect in this test. These results suggest that the pharmacological activities of medicinal plants are to be greatly affected by the extraction procedure, as in the case of *Valerianae Radix*, *Zizyphi Fructus*, *Psidium guajava* LINN. and *Rosmarinus officinalis* LINN.

Effects of partitioned fractions of MeOH extracts, 30% EtOH extracts and cacao extract

The results of partitioned fractions of the MeOH extracts, 30% EtOH extracts and cacao extract are shown in TABLES IV, V and VI, which show that the effective principles, except those of *Theobroma cacao* L. and *Psidium guajava* LINN., are more soluble in MeOH and aqueous solvent and that those of *Ginseng Radix* and *Valerianae Radix* are very soluble in water.

Toxicity

No mouse died when samples were injected at three times the doses used in the present experiment.

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