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Effects of Patriniae Radix and Melandrii Herba on Enzyme Activities in Mice

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Abstract—Effect of various fractions from the roots of *Patrinia scabiosaefolia* (Valerianaceae) and whole plants of *Melandryum firmum* (Caryophyllaceae) on enzyme activities in mice was investigated. The butanol fractions from both plants caused a significant elevation of serum transaminase activities when administered intraperitoneally, but did not, orally. Prolonged exposure by oral administration of both plants elevated hepatic cytochrome p-450 content, indicating the induction of drug metabolizing enzymes in liver.

Keywords—*Patrinia scabiosaefolia* · Valerianaceae · *Melandryum firmum* · Caryophyllaceae transaminase · alkaline phosphatase · cytochrome p-450

In the previous communications, it has been reported that the methanol extracts of several Chinese drugs caused a significant prolongation of hexobarbital-induced sleeping time and elevation of serum transaminase activities accompanied by severe histopathological changes in the hepatic tissues in mice.[1-3] These results strongly suggested that there may exist some hepatotoxic constituents in these plants.

In this study, the effect of various fractions from roots of *Patrinia scabiosaefolia* (Valerianaceae) and whole plants of *Melandryum firmum* (Caryophyllaceae) on hepatic drug metabolizing function as well as on serum transaminase activities was investigated.

† Part 2 in the series: Studies on Hepatotoxic substances in Medicinal Plants. For Part 1, see ref. 3.

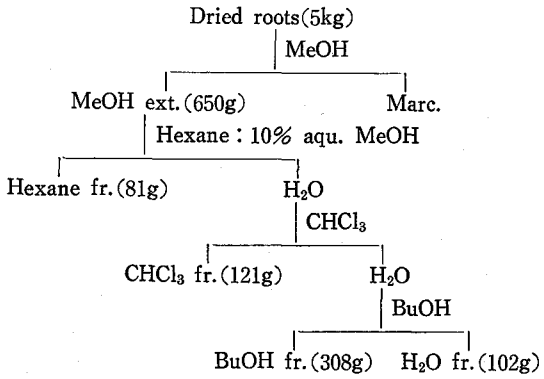
Experimental Methods

1. Plant material

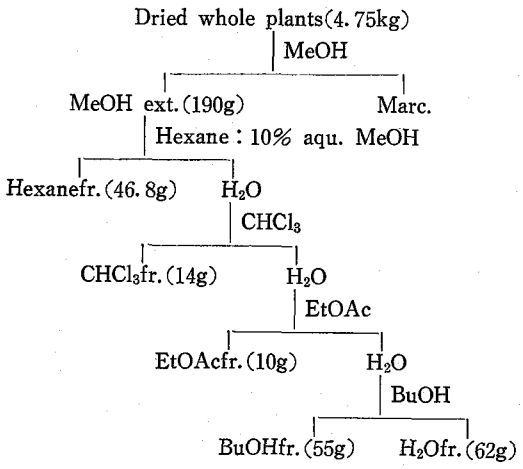
The roots of *P. scabiosaefolia* and the whole plants of *M. firmum* were collected in October in the mountain area near Seoul and botanically identified.

The dried plant materials were cut into small pieces and extracted three times with 90% MeOH on a water bath and concentrated to dryness. Each of the methanol extracts was fractionated as illustrated in scheme 1 and 2. Each fraction thus obtained was evaporated to dryness and rendered for the animal experiments.

The sprouts of both plants were also collected in the same area in April and May, air-dried and powdered, which were added to the normal



Scheme 1. Fractionation of the roots of *Patrinia scabiosaefolia.*



Scheme 2. Fractionation of the whole plants of *Melandryum firmum.*

diet at a level of 25% for feeding experiment.

2. Reagents

Reagent kits for determination of s-GOT, s-GPT and alkaline phosphatase activities were purchased from Abbott Laboratories Incorporation. Other reagents were first grade commercially available.

3. Animal pretreatments

Male dd mice weighing 20±3g were allowed lab chows and water *ad lib.* maintaining in a fixed temperature environment throughout the experiments. The indicated amount of test samples suspended in 0.5% carboxymethyl cellulose solution were administered intraperitoneally for three consecutive days or orally for 14 days to the experimental animals. The control animals were given vehicle only. Twenty four hr. after the last treatment, hexobarbital-induced sleeping time, serum enzyme activities and hepatic microsomal cytochrome p-450 content were measured.

In the second experiments, mice were fed lab chows containing powdered sprouts (25%) for 14 days and on the 15th day, the determination of serum enzyme activities and hepatic cytochrome p-450 level were carried out.

4. Measurement of hexobarbital induced sleeping time

Mice were injected i.p. with sod. hexobarbital (50 or 100mg/kg) and observed for sleep as evidenced by loss of the righting reflex. The duration of sleeping time was measured from the time of loss to the time the animals regained the righting reflex.

5. Measurement of serum enzyme activities

For the measurement of serum enzyme activities, mice were killed by cutting carotid artery and blood was collected carefully into a glass centrifuge tube and then allowed to clot in a refrigerator (4°) for approximately one hr. The serum was separated from the cells by centrifugation at 1000 rpm for 20 min and stored below 4° until the determination of enzyme activities in the same day. The GOT, GPT and alkaline phosphatase activities were estimated by measuring absorbance at 340nm for transaminases and at 415nm for alkaline phosphatase, respectively, after coupling the serum with corresponding reagents dissolved in purified demineralized water at 37° for 5 min using Automated Blood Analyzer (Abbott Laboratories, Model ABA-200).

6. Measurement of hepatic microsomal cytochrome p-450 content

Microsomal cytochrome p-450 content in liver was determined by the procedure of Omura and

Sato.[4]

Protein concentration was determined by the method of Lowry *et al.*[5] using bovine serum albumin as a standard.

Results and Discussion

The results of the effect of the fractions obtained from Patriniae radix and Melandrii herba on hexobarbital-induced narcosis which was estimated 24 hr after the 3 daily consecutive intraperitoneal pretreatments are shown in Table I and II.

Among four fractions from Patriniae radix, the butanol and chloroform fractions exhibited a marked prolongation in the duration of the barbiturate-induced narcosis in a dose response manner. The prolonging effect was shown to be the most pronounced in the group treated with the butanol fraction (Table I). In the case of Melandrii herba the chloroform and butanol fractions, too, elicited a significant prolongation of the barbiturate-induced narcosis. Even at a low dosage of the butanol fraction (10mg/kg, i.p.), 68% increase in sleeping time was observed

Table I. Effect of fractions from Patriniae radix on hexobarbital-sleeping time in mice

Treatment	Daily dose (mg/kg/day, i.p.)	Sleeping time[a] (min)	Percent of control
Control	—	69.4± 8.9	100
H_2O fr.	50	84.3± 12.7	121.5
	100	70.5± 12.1	101.6
Hexane fr.	50	99.9± 16.8	143.9
	100	85.1± 16.0	122.6
$CHCl_3$ fr.	50	143.6± 28.7**	206.9
	100	506.5±172.3**	729.8
BuOH fr.	50	421.5±170.0*	607.3
	100	798.9±236.1**	1,151.2

a) Mean±S.E. of 5 animals received 100mg/kg, i.p. of Na hexobarbital.
Significantly different from the control;
**$p<0.01$, *$p<0.05$

Table II. Effect of fractions from Melandrii Herba on hexobarbital-induced sleeping time in mice

Treatment	Daily dose (mg/kg/day, i.p.)	Sleeping time[a] (min)	Percent of control
Control	—	25.0± 1.2	100.0
Hexane fr.	50	31.2± 2.9	124.8
	100	40.0± 2.7*	160.0
H_2O fr.	—	—	—
EtOAc fr.	50	32.0± 2.8*	128.0
	100	37.2± 2.7**	148.8
$CHCl_3$ fr.	50	40.2± 2.1**	160.8
	100	42.0± 3.1**	168.0
BuOH fr.	100	died on 1st day	
	50	died on 2nd day	
	20	47.8± 1.7*	191.2
	10	42.0± 1.7*	168.0

a) Mean±S.E. of 5 animals received 50mg/kg, i.p. of Na hexobarbital.
Significantly different from the control;
**$p<0.01$, *$p<0.05$

(Table II). At a dose of 50mg/kg or more, the animals died within one or two days probably due to an acute toxicity.

Table III and IV shows the results of the effect of pretreatment of each fraction on serum GOT, GPT and alkaline phosphatase activities. CCl_4 (0.1ml/kg, i.p.) known as a typical hepatotoxic agent caused a remarkable increase in serum GOT and GPT activities as well as a significant increase in alkaline phosphatase activity (Table III).

In the case of Patriniae radix, all animal groups treated with the fractions except the water fraction exhibited a significant elevation in both GOT and GPT activities. The effect of the butanol fraction was the most pronounced as much the same % increase in enzyme activities could be observed at a half dosage of the chloroform fraction.

In the case of Melandrii herba, the animal groups treated with the chloroform and butanol fractions showed a significant increase in activ-

Table III. Effect of fractions from *Patriniae radix* on activities of serum transaminases and alkaline phosphatase in mice

Pretreatment	Daily dose (mg/kg/day, i.p.)	Serum enzyme activity(IU/ml)		
		GOT	GPT	Alkaline phosphatase
Control	—	208.9± 12.7	44.6± 1.7	149.3±10.0
Hexane fr.	100	423.9± 28.3**	144.6±30.0*	150.5±34.0
		(202.9)	(324.2)	(100.8)
H_2O fr.	100	258.8± 19.0	73.7± 9.3*	133.8±18.1
		(123.9)	(165.2)	(89.6)
$CHCl_3$ fr.	100	444.2± 43.4**	91.1±12.0**	156.4±36.9
		(212.6)	(204.3)	(104.8)
BuOH fr.	50	383.8± 38.0**	86.5± 8.3**	99.2±10.0
		(183.7)	(193.9)	(66.4)
CCl_4	0.1ml/kg	845.1±138.8**	504.8±99.8**	210.6±18.5*
		(404.5)	(1131.8)	(141.1)

Data are mean±S.E. of 5 or 6 animals. Figures in parentheses indicate % of the control.
Significantly different from the control; **$p<0.01$, *$p<0.05$

Table IV. Effect of fractions from *Melandrii herba* on activities of serum transaminases and alkaline phosphatase in mice

Pretreatment	Daily dose (mg/kg/day, i.p.)	Serum enzyme activity(IU/ml)		
		GOT	GPT	Alkaline phosphatase
Control	—	208.9±12.7	44.6±1.7	149.3±10.0
Hexane fr.	100	241.7±15.7	61.4±3.8*	170.0±12.3
		(115.7)	(137.7)	(113.9)
H_2O fr.	50	391.2±47.5**	56.0±3.9*	113.5±13.0
		(187.3)	(125.6)	(76.0)
$CHCl_3$ fr.	100	543.4±52.9**	94.1±5.0**	236.5±30.1*
		(260.1)	(210.9)	(158.4)
BuOH fr.	30	384.2±74.4*	88.4±2.4**	132.7±44.4
		(183.9)	(198.2)	(88.9)
EtOAc fr.	100	211.9±26.8	63.8±5.3**	168.6±12.9
		(101.4)	(143.0)	(112.9)

Data are expressed as mean±S.E. of 5 or 6 animals. Figures in parentheses indicate % of the control.
Significantly different from the control; **$p<0.01$, *$p<0.05$

ities of both transaminases. The effect of the butanol fraction was the most pronounced as well. The water and hexane fractions showed a small but significant elevation in the GPT activity only. No measureable alterations in alkaline phosphatase activity was shown by all the fractions from both plants except the chloroform fraction from Melandrii herba which caused 58.4% increase over the control.

Elevated activity in serum of the transaminases and other enzymes may reflect injury not only in the liver but other organs such as heart, skeletal muscle and kidney.[6,7]

The significant elevation of serum transamin-

Table V. Effect of repeated treatments of butanol fraction from *Patriniae radix* and *Melandrii herba* on transaminase activities and cytochrome p-450 content in mice

Treatment	Serum enzyme activity (IU/ml)		Hepatic cytochrome p-450 (nmoles/mg prot.)
	GOT	GPT	
Control	245.6± 15.9	92.7±10.3	0.46±0.04
Patriniae radix	221.8± 4.2	70.2± 8.2	1.06±0.08**
(1g/kg, p.o.)	(90.3)	(75.7)	(230.4)
Melandrii herba	271.8± 24.2	99.7±24.6	0.89±0.09*
(1g/kg, p.o.)	(110.7)	(107.6)	(193.5)
CCl_4	618.1±125.1*	449.4±6.4**	0.46±0.03
(0.025ml/kg)	(251.7)	(484.8)	(100.0)

Mice were treated orally daily for 14 days before sacrifice. Data represent mean±S.E. of 3 determinations. Figures in parentheses indicate % of control. Significantly different from the control; **$p<0.01$, *$p<0.05$

Table VI. Effect of sprouts of *P. scabiosaefolia* and *M. firmum* on serum transaminase activities and cytochrome p-450 content in mice

Pretreatment	Serum enzyme activity (IU/ml)		Hepatic cytochrome p-450 (nmoles/mg prot.)
	GOT	GPT	
Control (Normal Lab. Chows)	195.2±12.9	77.8± 9.4	0.37±0.02
Patrinia	216.6± 6.6	72.2± 6.0	0.80±0.06**
	(110.9)	(92.8)	(216.2)
Melandryum	202.3±25.2	88.6±18.7	0.91±0.15*
	(103.6)	(113.9)	(245.9)

Mice were fed lab chows containing 25% powdered dry sprouts for 14 days prior to sacrifice. Data are expressed as mean±S.E. of 5 or 6 animals. Figures in parentheses indicate % of the control. Significantly different from the control; **$p<0.01$, *$p<0.05$

ase activities induced by consecutive parenteral pretreatments of Patriniae radix and Melandrii herba was accompanied by fatty degeneration and Kupffer cell activation in liver cells,[3] which strongly indicated that it resulted from an acute hepatotoxicity caused by some toxic principles contained in these plants.

This assumption is strongly supported by the finding that the particular fractions of the plant extracts caused a significant prolongation of hexobarbital-induced sleeping time which might be resulted from the impairment of hepatic hexobarbital metabolism because of the acute liver dysfunction.[8] It is unlikely that the plants caused a cholestatic injury as there were no distinct alterations in alkaline phosphatase activity.[9]

In contrast to the effect of parenteral adminis tration, no significant elevation in GOT or GPT activities could be observed even when administered orally the butanol fractions at a dose as high as 1g/kg for 14 days as shown in Table V. Hepatic cytochrome p-450 level of butanol fraction-treated group, on the other hand, showed approximately two fold increase compared to those of the control, which indicated a significant induction of hepatic mixed function oxidase system.

In Korea, the sprouts of both plants are collected and commonly served as a vegetable dish. In order to clarify the effect of frequent ingestion of those sprouts on serum and hepatic enzymes, the dried powdered sprouts were mixed

with normal lab chows at a level of 25% and mice were fed for upto two weeks, and the serum and hepatic enzyme activities were estimated (Table VI).

In consistence with the effect of the oral administration of the butanol fractions the serum level of transaminases were also shown to be unaffected, whereas, a significant elevation of hepatic cytochrome p-450 level was shown in treated animal groups. Saponins have been isolated from the butanol fractions from both plants and demonstrated to be highly acutely toxic parenterally in the preliminary tests.[10] Saponins are generally known to be hardly absorbed when orally administered and some saponins are easily degraded in the gastro-intestinal tract.

From the discrepant findings with respect to the route of the administration in the present experiment, it can be postulated that one of major hepatotoxic principles might be saponins, which will undergo degradation in the gastrointestinal tract into more easily absorbable smaller moiety such as prosapogenin or genins.

The gradual absorption of these degradation products might provoke induction of hepatic mixed function oxidase system eventually leading to untoward side effects.

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