

Central Depressant Effects of the Extracts of Magnolia Cortex¹⁾

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Pharmacological properties of the extracts from the magnolia cortex (the bark of *Magnolia obovata* THUNB.) were examined with special reference to their central nervous system action. The ether extract of the magnolia (1 g/kg *i.p.*) markedly depressed the spontaneous activity of mice and chicks, while the water extract (1 g/kg *i.p.*) produced prompt paralysis of respiration. Distinct muscle weakness was found after administration of the ether extract, through the clinging power test on the wire net, and was different from the effect of the water extract. The ether extract suppressed convulsion produced by strychnine, picrotoxin, or pentetrazol. Tremor by oxotremorine was also blocked by the ether extract. Pentetrazol infusion technique was employed for the elucidation of the mode of this drug action. Depressive effect of the ether extract was also exerted on the crossed extensor reflex of the chick spinal cord.

Magnolia cortex (the bark of *Magnolia obovata* THUNB., "Wakoboku" in Japanese) is an important component of the crude drug prescriptions in Kanpo medicine (Chinese traditional medicine ancestrally inherited and developed in Japan). The traditional prescriptions containing magnolia cortex are dosed for gastro-intestinal disturbance or anxiety.

A few modern Kanpo clinicians³⁾ tried to use this crude drug for the therapy of Parkinsonism and reported successful results. Nagahama⁴⁾ stated that the clinical trial had been performed on the basis of the finding that magnolia cortex contained curare-like substances. The curare-like activity of magnolia cortex was found by Sasaki⁵⁾ in 1921, and its active principles have been elucidated by Tomita, Inubushi, and their collaborators.⁶⁾ We, however, considered that peripheral curare-like effect was not sufficient to explain the beneficial effect of this crude drug against Parkinsonism, and we assumed that magnolia cortex might have some central effect in addition to curare-like action.

In this paper, we describe the findings that ether extract of magnolia cortex has a distinct central nervous system-depressing effect *i.e.*, sedation, loss of righting reflex, centrally acting muscle relaxation, depression of drug-induced convulsion and tremor, and spinal reflex inhibition.

Material and Method

Preparation of the Extracts—The bark of *Magnolia obovata* THUNB. from a commercial source⁷⁾ was sliced with a plane, and separately extracted with three kinds of purified solvent, water, ethanol, and ether, at each of their boiling point for 2 hr. In each extraction procedure, 50 g of the bark was treated with 500 ml of the solvent. After the solvent was almost evaporated, remaining solvent in viscous residue was

- 1) A part of this work was presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
- 2) Location: 3190, Gofuku, Toyama, 930, Japan.
- 3) K. Ohtsuka, D. Yakazu, and T. Shimizu, "Kanpo Shinryo Iten" (Textbook for Kanpo Clinics), Nanzando, Tokyo, 1969, p. 160.
- 4) Y. Nagahama "Toyo Igaku Gaisetsu" (An Introduction to Oriental Medicine), Sogensha, Osaka, 1968, p. 278.
- 5) T. Sasaki, *Fukuoka Med. J.*, **14**, 391 (1921).
- 6) M. Tomita, Y. Inubushi, and M. Yamagata, *Yakugaku Zasshi*, **71**, 1069 (1951); K. Ito and A. Yoshida, *Yakugaku Zasshi*, **86**, 124 (1966).
- 7) We are grateful to Dr. T. Namba and Dr. M. Yoshizaki for their kindness in identifying the crude drug.

carefully eliminated. The yield of each extract on an average was as follows: Water extract, 14%; ethanol extract, 12%; and ether extract, 8%. For the pharmacological experiments, the water extract was dissolved in physiological saline solution, and the ether or ethanol extract was suspended in the vehicle containing 0.5% of carboxymethyl cellulose (CMC) and 10% of Tween 80.

General Behavior—Male mice (ddY strain, 20–25 g, from Shizuoka Experimental Animal Nokyo) and young chicks (male Nick Chick Leghorns, 30–40 g, three days after hatching, from Hokuriku H & N Farm) were used. Each extract was administered to the test animal in a dose of 1 g/kg intraperitoneally or 2 g/kg orally. General behavior of mice was observed for 6 hr, and any notable symptoms were recorded according to the criteria suggested by Lim.⁸⁾ The behavior of young chicks was also observed.

Test for Muscle Relaxation—Muscle weakness of mice after drugs administration was measured in terms of duration of clinging on overhanging wire net. The effect of mephenesin was also examined as the control drug. Other materials and methods were the same as those in the case of general behavior observation.

Anticonvulsant Activity—Spontaneous activity and drug-induced convulsion or tremor of mice were recorded by using a simple kymographic recording apparatus as described by Fukuda, *et al.*⁹⁾ A mouse was mounted in a cage (5 × 8 × 4 cm), the movement of which was recorded on a smoked drum by a writing lever connected to the cage through an oscillation board.

A convulsant or a tremor-inducing drug was injected subcutaneously 50 min after intraperitoneal administration of the ether extract of the magnolia bark (1 g/kg).

Pentetrazol Infusion—Dose dependency of anticonvulsant effect of the magnolia extract was examined by the method described by Bastian, *et al.*¹⁰⁾ with a slight modification. Male mice weighing 20–25 g were used. Pentetrazol, 10 mg/ml in saline, was infused at the rate of 0.12 ml/min through a 40-cm polyethylene tube connected with a needle of 0.2 mm in diameter which was inserted into the tail vein. A fixed infusion rate was maintained with a motor-driven apparatus (Natume KN201H). The infusion was stopped at 300 sec or at death of animal. Four stages of the symptoms described by Orloff, *et al.*¹¹⁾ were taken as the checkpoint of the drug effect, *i.e.*, first twitch, pseudoconvulsion, persistent convulsion, and death. Infusion of pentetrazol was started 50 min after the intraperitoneal injection of the magnolia extract.

Spinal Reflex in Young Chicks—Preparation and methods of recording the spinal reflex were the same as described by Fukuda, *et al.*¹²⁾ Chicks ranging from 4 to 13 days in age were used. In a chick anesthetized with chloralose (50 mg/kg *i.p.*), the trachea was cannulated to avoid suffocation from hypersialosis. Crossed extensor reflex of the leg was elicited by stimulation of contralateral peroneal nerve (supramaximal square wave pulses, 0.2 cps, 1 msec in duration from the electronic stimulator, Nihon Kohden MSE-20). Ipsilateral flexor reflex of the anterior tibial muscle was also elicited. Reflexes were recorded on a kymograph through an isotonic lever. Drugs were administered intraperitoneally.

Result

General Behavior

Protocols of the observations on five mice for each treatment are summarized in Fig. 1 according to the schematic expression of animal behavior described by Lim⁸⁾ with a slight modification for mice.

Effect of Water Extract of Magnolia Bark—After intraperitoneal injection of the water extract (1 g/kg), the mice showed retching-like behavior in 15–20 sec and fell into a marked muscle weakness in 2–3 min. Breathing stopped soon after the muscle weakness was observed but at that instant the heart was found beating, then it stopped in a few minutes. In contrast with the strong lethal effect of intraperitoneally injected water extract, orally administered water extract (2 g/kg) did not show any remarkable effect other than a slight decrease in spontaneous movement of a short duration.

Effect of Ether Extract of Magnolia Bark—Spontaneous movement of a mouse gradually decreased 10 min after intraperitoneal injection of the ether extract of the magnolia bark

8) R.K.S. Lim, "Pharmacologic Techniques in Drug Evaluation," ed. by J.H. Nodine and P.E. Siegler, Year Book Medical Publishers Inc., Chicago, 1964, p. 294.

9) H. Fukuda, K. Watanabe, N. Nakamura, C. Hoshino, K. Mizukami, and K. Takatori, *Yakugaku Zasshi*, **89**, 354 (1969).

10) J.W. Bastian, W.E. Krause, S.A. Ridlon, and N. Ercoli, *J. Pharmacol. Exptl. Therap.*, **127**, 75 (1959).

11) M.J. Orloff, H.L. Williams, and C.C. Pfeiffer, *Proc. Soc. Exptl. Biol. Med.*, **70**, 254 (1949).

12) H. Fukuda, K. Watanabe, Y. Kudo, and T. Oshima, *Yakugaku Zasshi*, **88**, 1338 (1968).




































Extract	Water		Ethanol		Ether		Vehicle
Dose (g/kg)	1.0	2.0	1.0	2.0	1.0	2.0	10ml/kg
Route	<i>i.p.</i>	<i>p.o.</i>	<i>i.p.</i>	<i>p.o.</i>	<i>i.p.</i>	<i>p.o.</i>	<i>i.p.</i>
Before							
After 20min							
40min							
1 hr							
2 hr							
3 hr							

Fig. 1. Effect of Magnolia Extracts on Mouse Behavior

Schematic expressions of depressive state were cited from Lim⁹⁾ with a slight modification. Five animals were observed for each treatment and the approximate average of the time for behavior changes is expressed in the schematic pictures.

(1 g/kg). The mouse took a depressed posture with falling head and abdomen on the floor 15–20 min after the injection, but it easily awakened in response to stimulation. After 25 min, marked depression with muscle relaxation was produced, and righting reflex was lost. Such a depressed state continued for about 50 min, and then the righting reflex returned. In about 4 hr after the injection, the mouse almost recovered from the depression. Some mice were found dead in the cage next morning, even though they had recovered from the depression. Almost the same effect was observed in the experiment with young chicks, all of which survived over three days. Orally administered ether extract (2 g/kg) also showed a marked depressive effect in mice. Although loss of righting reflex was not observed with the oral dose, characteristics of the depression were similar to those with intraperitoneal administration.

Effect of Ethanol Extract of Magnolia Bark—Effect of intraperitoneally injected ethanol extract (1 g/kg) was intermediate between the water and ether extracts. Two out of five mice died from respiratory inhibition after 20 min, showing profound depressive symptoms before death. Other animals behaved similarly to those receiving the ether extract intraperitoneally except the absence of the loss of righting reflex. Distinct depression was observed also by oral administration of the ethanol extract (2 g/kg). Effect of the vehicle was examined and found to be negligible.

Dose Dependency of the Action of Each Extract on General Behavior—Depressive action on motor function and lethal effect of each extract in various doses are shown in Table I. With the water extract (1 g/kg *i.p.*), animals died from respiratory failure as soon as they lost the righting reflex, and the dose range between the survival and the lethal effect was assumed to be rather narrow (between 0.84 and 1.0 g/kg). In contrast with the water extract, the ether extract produced loss of righting reflex without lethal effect. The ethanol extract showed the intermediate action between the water and the ether extract.

Muscle Relaxing Activity

Duration of clinging on the overhanging wire net was measured after the administration of each extract of the magnolia bark or mephensesin, and the results are shown in Fig. 2.

TABLE I. Effect of Magnolia Extracts on Mouse Behavior

Treatment		Loss of righting reflex	Death within 3 hr
Extract	Dose (g/kg <i>i.p.</i>)		
Water	0.71	0/10	0/10
	0.84	1/10	1/10
	1.00	10/10	10/10
Ethanol	1.00	4/10	4/10
	1.41	7/10	7/10
	2.00	10/10	10/10
Ether	0.50	0/10	0/10
	0.71	4/10	0/10
	1.00	10/10	0/10

Soon after the intraperitoneal administration of the water extract (1 g/kg), mice lost their muscle tone and died within a few minutes, while no change of behavior was observed after oral administration even in double the dose (2 g/kg). This finding indicates that the muscle relaxing effect of the water extract may be a curare-like action. In the case of the ether extract, both oral and intraperitoneal administration were effective in showing distinct relaxation, and the action lasted over 3 hr. The mode of the muscle-relaxing action of the ether extract seemed quite different from that of the water extract. The former never exerted a lethal effect on mice in spite of strong muscle relaxation with the tested dose. The ethanol extract showed an intermediate character between the ether and the water extract. Intraperitoneal administration of the ethanol extract (1 g/kg) produced a distinct muscle relaxation, but the effect of oral dose (2 g/kg) of the ethanol extract was much weaker than that of the ether extract. The effect of mephenesin (250 mg/kg *i.p.*) was found to be shorter in duration of action than the ether extract. No change in the muscle tone was observed after intraperitoneal administration of the vehicle containing 10% of Tween 80 and 0.5% of carboxymethyl cellulose (CMC).

Antagonistic Action against Convulsants

After the pretreatment of mice with the ether extract of the magnolia bark (1 g/kg *i.p.*, 50 min before a convulsant), strychnine (1 mg/kg *s.c.*) did not produce any convulsion in mice (Fig. 3-B), while without the pretreatment all the mice died showing the characteristic convulsive symptoms within 10 min (Fig. 3-A).

Picrotoxin (7.5 mg/kg *s.c.*) showed its strong convulsive action in 20 min and the mice died after tonic convulsion (Fig. 4-A). After the pretreatment with the ether extract, no convulsive symptoms were observed and none of the mice died for over 3 hr, but some animals showed tachypnea (Fig. 4-B).

The antagonistic action of the ether extract of magnolia bark was not so complete with pentetrazol (120 mg/kg *s.c.*) as against strychnine or picrotoxin. By treatment with the

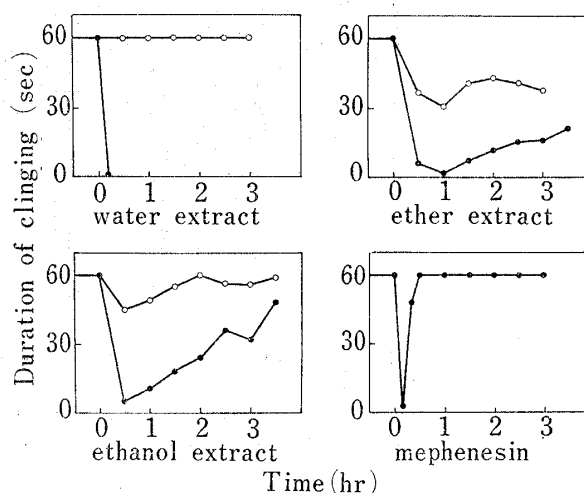


Fig. 2. Muscle Relaxing Activity of Magnolia Extracts

Each point expresses the mean value for eight animals. —○—: Magnolia extract (2 g/kg *p.o.*), —●—: magnolia extract (1 g/kg *i.p.*) or mephenesin (250 mg/kg *i.p.*) Duration over 60 sec was plotted on the same position as 60 sec.

ether extract (1 g/kg *i.p.*), both extensor tonus and death were prevented but the convulsive twitch was observed even 30 min after the pentetrazol (Fig. 5).

The tremor induced by oxotremorine (1 mg/kg *s.c.*) was almost completely inhibited by pretreatment with the ether extract (1 g/kg *i.p.*) (Fig. 6). In this case sialosis by oxotremorine was not inhibited.

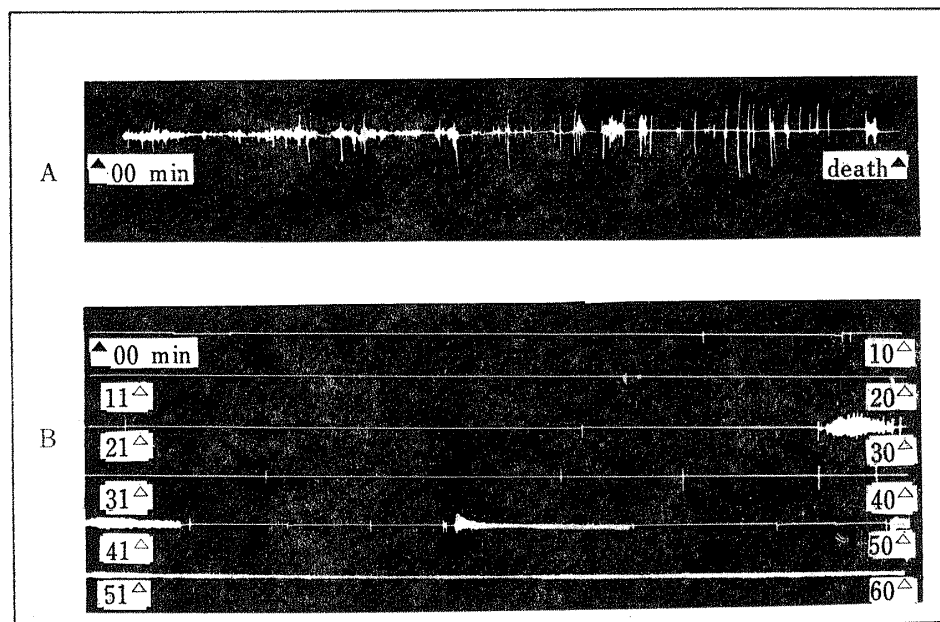


Fig. 3. Anticonvulsant Effect of the Ether Extract of Magnolia Bark against Strychnine

Kymographic recordings of the movement of the mouse in a small cage. The figures in the records indicate the time after the stimulant injection in minutes. The magnolia extract was injected 50 min before the stimulant.

A: strychnine nitrate (1 mg/kg *s.c.*) without pretreatment;

B: Strychnine after pretreatment with the ether extract of the magnolia bark (1 g/kg *i.p.*).

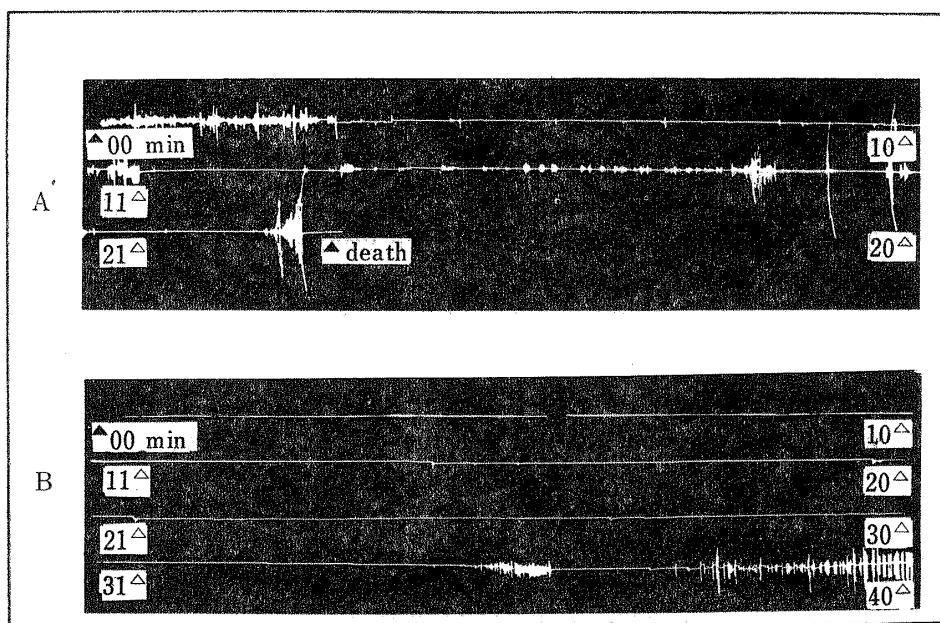


Fig. 4. Anticonvulsant Effect of the Ether Extract of Magnolia Bark against Picrotoxin

The indications are the same as in Fig. 3.

A: picrotoxin (7.5 mg/kg *s.c.*) without pretreatment;

B: Picrotoxin (7.5 mg/kg *s.c.*) after pretreatment with the ether extract of magnolia bark (1 g/kg *i.p.*).

Pentetrazol Infusion

As described by Orloff, *et al.*,¹¹⁾ easily recognizable four symptoms, first twitch, pseudo-convulsion, persistent convulsion, and death, were observed during the pentetrazol infusion through the tail vein of mice. In order to elucidate the dose dependency of the anticonvul-

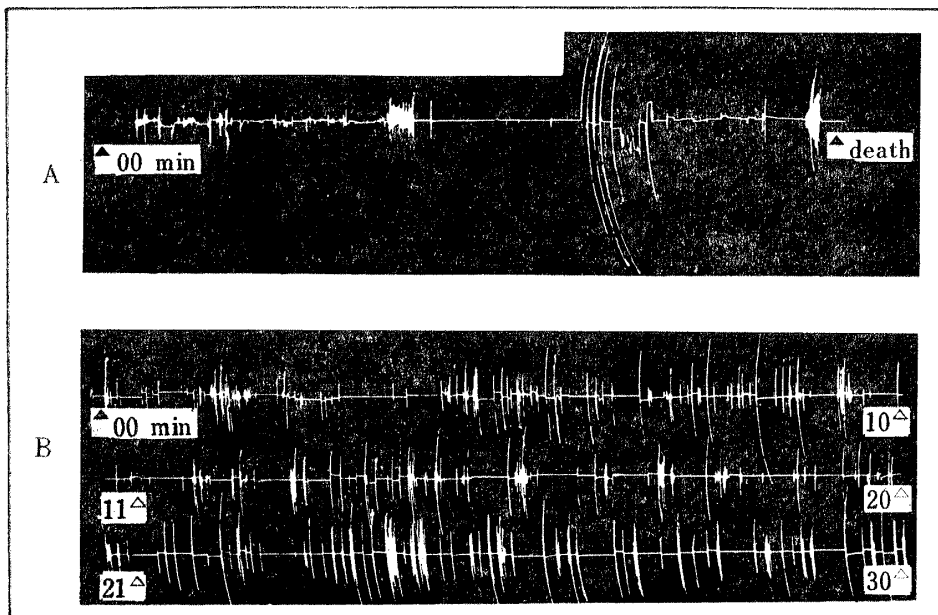


Fig. 5. Anticonvulsant Effect of the Ether Extract of Magnolia Bark against Pentetrazol

The indications are the same as in Fig. 3.

A: pentetrazol(120 mg/kg s.c.) without pretreatment;

B: Pentetrazol (120 mg/kg s.c.) after pretreatment with the ether extract of magnolia bark (1 g/kg i.p.).

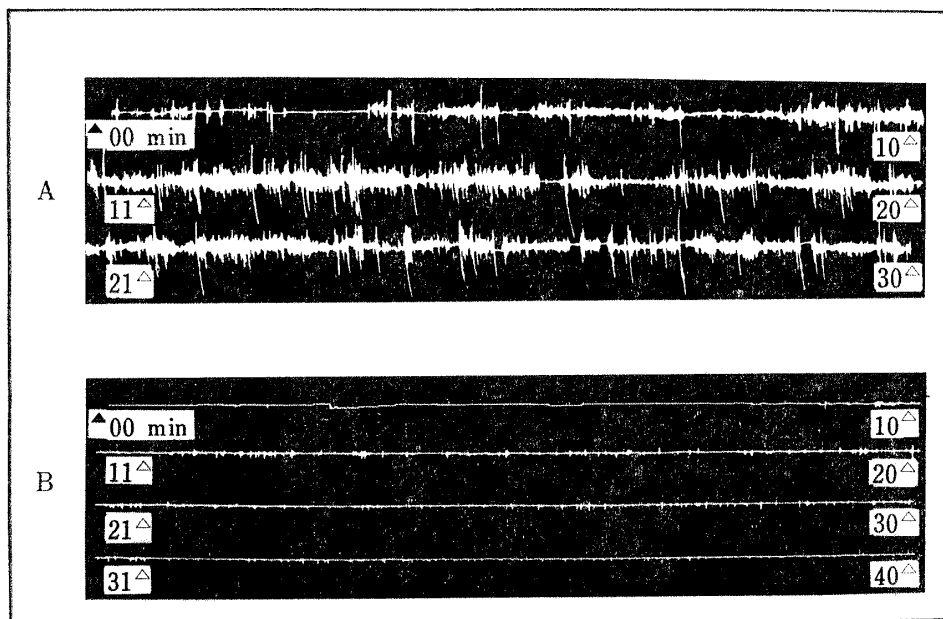


Fig. 6. Depressive Effect of the Ether Extract of Magnolia Bark on Oxotremorine Tremor

The indications are the same as in Fig. 3.

A: oxotremorine (1mg/kg s.c.) without pretreatment;

B: Oxotremorine (1 mg/kg s.c.) after pretreatment with the ether extract of magnolia bark (1 g/kg i.p.).

sant effect of the ether extract of magnolia bark, three graded doses were examined for its antagonistic action on the convulsive symptoms. As shown in Table II, the time between the start of infusion and death was closely related to the dose of the extract. However, the inhibitory action against persistent convulsion was not proportional to the dose, even though the anticonvulsive effect was observed clearly. First twitch was quite resistant to the magnolia extract.

TABLE II. Effect of the Ether Extract of Magnolia Bark on Convulsion Symptoms induced by Pentetrazol Infusion

Treatment	First twitch	Pseudo-convulsion	Persistent convulsion	Death
Control (vehicle alone)	52.0 ± 4.7 ^{a)}	64.3 ± 8.1	98.8 ± 10.9	156.6 ± 40.0
Ether extract				
0.5 g/kg <i>i.p.</i>	45.3 ± 1.7	64.0 ± 5.8	193.3 ± 23.3	394.5 ± 134.2
0.71 g/kg <i>i.p.</i>	61.3 ± 11.0	86.3 ± 16.3	180.8 ± 37.9	623.0 ± 169.9
1.0 g/kg <i>i.p.</i>	56.5 ± 4.0	120.0 ± 13.7	^{b)}	>900
Mephesisin 400 mg/kg <i>p.o.</i>	58.0 ± 7.2	94.4 ± 13.8	192.0 ± 23.4	394.0 ± 128.6

a) Each value represents the mean time (in sec) with standard error after the start of infusion.
b) Persistent convulsion was completely blocked.

Inhibition on Spinal Reflex in Young Chicks

Intraperitoneal injection of the ether extract of the magnolia bark (1 g/kg *i.p.*) inhibited the crossed extensor reflex of chicks (Fig. 7-A). The inhibition was observed from about 20 min and reached its maximum effect about 50 min after the injection. This inhibition

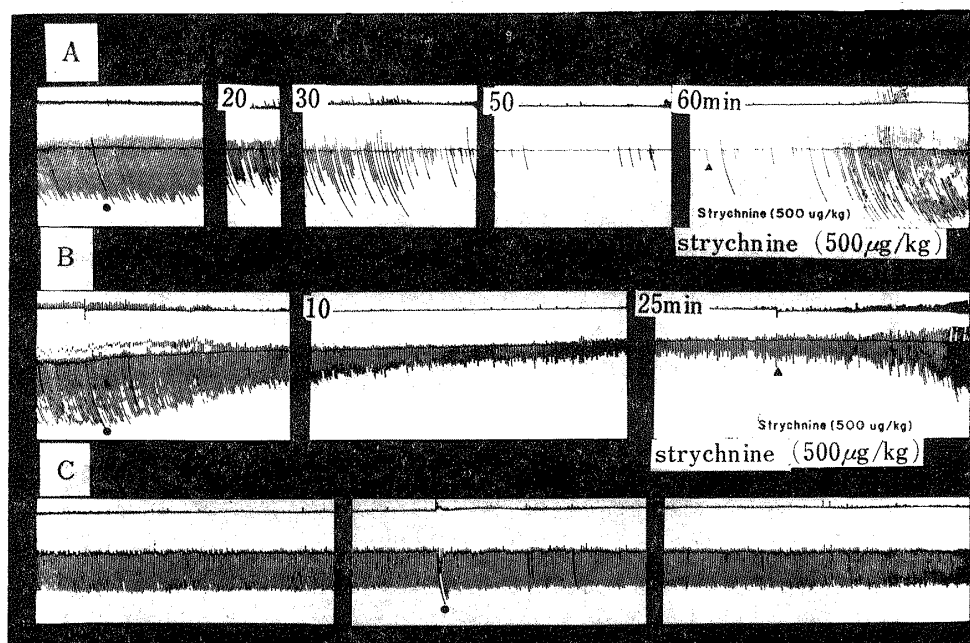


Fig. 7. Effect of the Ether Extract of Magnolia Bark on Spinal Reflexes in Young Chicks

Kymographic recordings of the crossed extensor reflex of the leg (lower traces) and the flexor reflex of anterior tibial muscle (upper traces) induced by electrical stimulation on the peroneal nerve.

A: The ether extract of the magnolia (1 g/kg *i.p.*);

B: mephesisin (200 mg/kg *i.p.*);

C: control (vehicle containing 10% Tween 80 and 0.5% CMC)

was antagonized by strychnine (500 $\mu\text{g}/\text{kg}$ *i.p.*), suggesting that the action of the magnolia ether extract was not a curare-like peripheral effect. The crossed extensor reflex responded to the stimulation of the central end of the cut sciatic nerve was inhibited by the ether extract, whereas the contraction of the ipsilateral anterior tibial muscle induced by the stimulation of the peripheral end was not inhibited. This observation also supports the above suggestion. Mephenesin (200 mg/kg *i.p.*) was found to have a marked inhibitory action on the spinal reflex, and this effect was also antagonized by strychnine (500 $\mu\text{g}/\text{kg}$ *i.p.*) (Fig. 7-B). The vehicle did not show any action on the spinal reflex (Fig. 7-C).

Discussion

Chemical⁶⁾ and pharmacological¹³⁾ studies on the magnolia cortex have been focussed on the alkaloidal components since Sasaki⁵⁾ found a curare-like substance, and some of the therapeutic effects of this drug were ascribed to these alkaloids.⁴⁾ However, the traditional therapeutic use of this drug for anxiety or neuronal disturbances suggests that the effect of this drug should be due to its action on the central nervous system.

In the first series of the present experiments, we found that the ether-soluble fraction had a central depressive action. The known constituents in this fraction are an essential oil¹⁴⁾ and phenolic compounds,¹⁵⁾ but the pharmacological action of these compounds has never been reported.

The characteristic property of the central nervous system-depressing action of the magnolia extract is slow in onset and is long lasting. It may be due to the slow rate of absorption of the suspended oily substance, since the effect of subcutaneous injection was much weaker than intraperitoneal or even than oral administration.

Even though the mice lost righting reflex after administration of the ether extract, its central-depressing effect was not so complete as by general anesthetics, since they could take a normal position, with effort, in response to strong pain stimulus.

Muscle weakness due to the ether extract was so distinct that the duration of clinging on the wire net was almost a few seconds in average at the peak of the action. Muscle relaxing effect was observed also after oral administration of the ether extract, and its effect was distinguished from that of the water extract. The water extract showed a strong muscle relaxing action with prompt onset and lethal effect caused by a respiratory failure. However, oral administration of the water extract did not exert any action other than a weak sedation.

Central depressing action of magnolia bark extract was also proved by its inhibitory effect against various kinds of convulsants. This inhibitory action seemed to be nonspecific. Pentetrazol infusion technique provided further information on the mode of action of the ether extract, which elevated the threshold of death and persistent convulsion of mice by the infused pentetrazol, but slightly affected the first twitch and pseudoconvulsion. Bastian, *et al.*¹⁰⁾ classified the central depressants on the basis of their mode of action against pentetrazol infusion. According to their classification, the action of the ether extract seems to belong to the mephenesin group.

The ether extract inhibited the tremor induced by oxotremorine, but did not prevent sialosis. This effect may be due to a nonspecific central action or centrally acting muscle relaxing effect.

The action of the ether extract on the spinal reflex was also examined in chicks. The crossed extensor reflex induced by electrical stimulation on the peroneal nerve was distinctly

13) K. Ogiu and M. Morita, *Nippon Yakurigaku Zasshi*, **48**, 72 § (1952); K. Miki, *Nippon Toyo Igaku Zasshi*, **4**, 17 (1953); K. Ogiu, H. Takagi, and M. Morita, *Nippon Yakurigaku Zasshi*, **51**, 209 (1955); K. Inoue, *Nippon Yakurigaku Zasshi*, **53**, 797 (1957).

14) Y. Sugii and H. Shindo, *Yakugaku Zasshi*, **50**, 709 (1930).

15) Y. Sugii, *Yakugaku Zasshi*, **50**, 183 (1930); M. Fujita, H. Itokawa, and Y. Sashida, *Chem. Pharm. Bull.* (Tokyo), **20**, 212 (1972).

inhibited. This action was differentiated from the curare-like effect because a high dose of strychnine restored the reflex. Furthermore, the ether extract did not inhibit the contraction of the anterior tibial muscle responded to the stimulation on the peripheral end of the cut sciatic nerve in the ipsilateral leg, whereas the crossed extensor reflex elicited by the stimulation on the central end was completely inhibited. This fact may be regarded as the evidence indicating that the spinal reflex inhibition by this extract is not due to a curare-like peripheral action.

Elucidation of the active principles in the ether extract of the magnolia cortex is left for future study.