

PHARMACOLOGICAL STUDIES ON ROOT BARK OF MULBERRY TREE (*Morus alba* L.)

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Accepted April 23, 1976

Abstract—Pharmacological studies were done on the root bark of mulberry tree and pharmacological effects were compared with the clinical effects of “Sohakuhi” in Chinese medicine. *n*-Butanol- and water-soluble fractions of mulberry root had similar effects except for those on the cardiovascular system. Both fractions showed cathartic, analgesic, diuretic, antitussive, antiedema, sedative, anticonvulsant, and hypotensive actions in mice, rats, guinea pigs and dogs. There appears to be a correlation between the experimental pharmacological results and the clinical applications of mulberry root found in the literature on Chinese medicine.

Mulberry leaves have been widely cultivated in China and Japan as indispensable food of silkworms. On the other hand, the root bark of mulberry tree, called “Sohakuhi”, has been used medicinally since olden times. It is said in Chinese medicine that Sohakuhi has antipyretic, antitussive, expectorant, diuretic and laxative activities (1-2). In addition, Sohakuhi has been said to prevent palsy. Chemical research on mulberry root has been reported by Uno (3), Carruthers, et al. (4), and Oku (5). According to these authors, mulberry root contains sterols, flavon, flavanone, stilbene, benzophenone and coumarine derivatives.

In the pharmacological field, few papers concerning the extracts of mulberry root have been reported. Oishi (6), Fukutome (7), Suzuki, et al. (8), and Tanemura (9) reported hypotensive effects of mulberry root. It was suggested that the hypotensive component would probably be acetylcholine or its analogues (9).

In our study, pharmacological activities of mulberry root were widely investigated and the correlation between laboratory results and clinical applications is discussed.

MATERIALS AND METHODS

Extracts of mulberry root bark were prepared as shown in Fig. 1. Both water soluble and *n*-butanol soluble fractions (W- and B-fractions, respectively) of mulberry root bark were dark brown powder and were soluble in water. Both fractions were used in the following pharmacological tests. They were dissolved in physiological saline and neutralized by sodium carbonate solution.

Acute toxicity in mice

Male mice (dd-strain), weighing 18-22 g, were given oral, i.p. and i.v. administrations and mortality was determined 72 hr after.

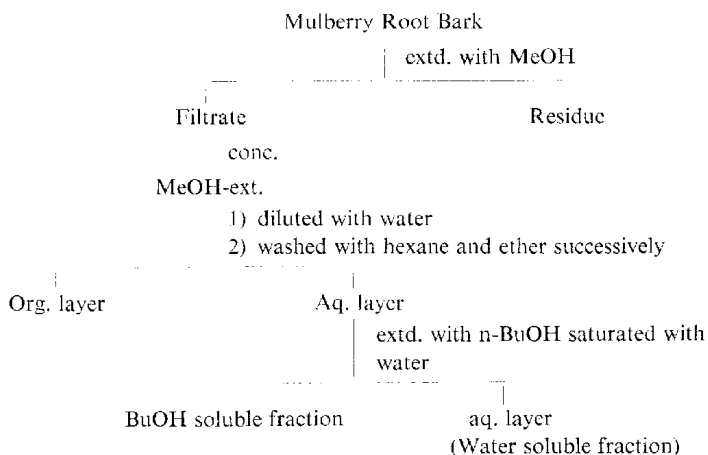


FIG. 1. Preparation of the Extracts of Mulberry Root Bark.

Behavioral observations in mice

Neuropharmacological observations in mice were made after Irwin's method (10). Groups of 3 male mice, weighing 18–22 g, were given the test substance i.p., and observed every 30 min for 2 hr.

Anticonvulsion test in mice (Electroshock method)

Groups of 10 male mice, weighing 20–24 g, were given the test substance i.p., and 30 min later were subjected to the maximum electroshock (corneal electrodes with 50 mA and 0.1 sec).

Analgesic tests in mice

Writhing induced by 1% acetic acid: Male mice in groups of 10, weighing 18–22 g, were given the test substance orally, and 30 min later an i.p. injection of acetic acid. The number of writhing responses per mouse was recorded for a period of 10 min, beginning 10 min after administration of acetic acid.

Tail pressure test: The method used was that described by Takagi, et al. (11). Groups of 8 male mice, weighing 18–22 g, were tested after an i.p. administration of a test substance.

Hypothermia in rats

Groups of 6 Wistar male rats, weighing about 180 g with 37.5°–38.9°C rectal temperature, were given the test substance orally, and rectal temperature was recorded every 1 hr for 4 hr at a room temp. of 23°C.

Anti-inflammatory tests in rats (Carrageenin- and dextran-induced edema)

Groups of 4 Wistar male rats, weighing about 120 g, were given the test substance orally, and 30 min later an s.c. injection of 1% carrageenin or 6% dextran solution in volume of 0.1 ml in the hind paw. The volume of the foot was recorded every 1 hr for 5 hr.

Tests on the cardiovascular system in dogs and rats

In anesthetized dogs: Nine male mongrel dogs anesthetized with pentobarbital, 30 mg/kg i.v., were used. Femoral arterial pressure and heart rate were measured. In two

dogs, gastric and ileal movements were also recorded with water balloons. In 4 dogs, effects of i.a. injections of test substances on brachial blood flow were also investigated using an electromagnetic flow meter.

In anesthetized rats: Groups of 5 Wistar male rats anesthetized with urethane, 1.2 g/kg i.p., were used. Femoral arterial pressure and heart rate were measured.

On the isolated rat atria: Spontaneous beating of the isolated atria and electrically driven (3 msec, 0.5 Hz) contractions of isolated left atrium mounted in oxygenated Krebs-Henseleit II solution at 37°C were isometrically recorded.

Tests on the isolated smooth muscle preparations (Guinea pig ileum, tenia caecum and vas deferens, and rat antrum and vas deferens)

Male guinea pigs weighing about 400 g and Wistar male rats weighing about 350 g were sacrificed by a blow on the head. Isolated preparations were suspended in Tyrode solution bubbled with air in the guinea pig ileum and the rat antrum or with 95% O₂-5% CO₂ in the others in a 20 ml organ bath kept at 30 ± 2°C. The responses were recorded isotonicly on a smoked paper. Rat antrum was prepared by Ueda's method (12).

Diuretic activity in rats

The renal actions of test substances were studied in male conscious hydrated rats (Wistar strain, 200 g b.w.). Urine was collected every 1 hr for 4 hr following a p.o. or i.p. administration of a test substance, and analysed for Na, K and Cl ion contents with a flame-photometer and Sino-test papers.

Intestinal propulsion test in mice

Groups of 8 male mice (dd-strain), weighing 18-22 g, were given a test substance, and 1 hr later BaSO₄ solution orally. Twenty min after the administration of BaSO₄, the animals were sacrificed and the transit length of BaSO₄ in the intestine was measured.

Cathartic test in mice

The method used was that described by Tsukui, et al. (14). Male dd-strain mice, weighing about 20 g, were placed on filter papers. Mice with normal feces were selected for the test, and given the test substance orally twice every 3 hr. Three hr after each administration, conditions of feces were observed. Diarrhea was scored in the following manner: normal (0), swelling (0.5), and fluid-like feces (1.0).

Antitussive test in guinea pigs

Male guinea pigs, weighing about 300 g were anesthetized lightly with thiopental. The method used was that described by Takagi, et al. (13).

Corneal anesthesia in guinea pigs

Male guinea pigs, weighing about 280 g, were used to study local anesthetic properties. Anesthetic activity was observed after exposure of the cornea to a test substance solution.

RESULTS

Acute toxicity in mice

Administration of 2, 5 and 10 g/kg of both fractions given either i.p. or p.o. and 2 and 5 g/kg given i.v. caused no death in each group of 3 male mice. Lethal doses of the fractions

were therefore more than 5 (i.v.), 10 (i.p.) and 10 g/kg (p.o.). These high doses produced a depression of locomotor activity as exhibited by a decrease in spontaneous movement accompanied with crouching posture.

Behavioral observations in mice

Both fractions up to 50 mg/kg i.p. had no significant effect on the gross behavior. Higher doses produced the following common symptoms for 2 hr after administration: decreases in alertness, visual placing, passivity, grooming, and spontaneous movement; slight decreases in touch and pain responses, and body tone; a slight increase in pupil size. Abnormal gait was also observed. A decrease in body temperature was observed after the administration of B-fraction, and piloerection after that of W-fraction. The effects of B-fraction on behavior were in general more significant than those of W-fraction.

Anticonvulsion test in mice (Electroshock method)

As shown in Table 1 both fractions had a mild inhibitory effect on electroshock.

TABLE 1. Effect of mulberry root on maximal electroshock seizure in mice

Treatment	Dose (i.p.)	No. of animals	No. of TE ^c	No. of deaths
Saline		10	10	10
B-fraction ^{a)}	1 g/kg	10	10	9
	3	10	10	6
	10	10	10	1
W-fraction ^{b)}	1	10	10	6
	3	10	9	6
	10	10	10	2
Phenobarbital	30 mg/kg	10	0	0

a) B-fraction: n-Butanol soluble fraction of mulberry root.

b) W-fraction: Water soluble fraction of mulberry root.

c) TE: extensor tonus.

Analgesic tests in mice

Writhing induced by 1% acetic acid: As shown in Fig. 2, W-fraction inhibited writhing responses significantly, as to some extent did B-fraction in a dose of 2.0 g/kg p.o.

Tail pressure test: As shown in Fig. 3, both fractions tended to increase the maximum pain threshold in a dose of 2.0 g/kg p.o.

Hypothermia in rats

Neither fraction had a significant effect on body temperature in rats.

Anti-inflammatory tests in rats (Carrageenin- and dextran-induced paw edema)

As shown in Table 2, W-fraction in doses of 3.0 and 5.0 g/kg p.o. significantly inhibited carrageenin edema while such inhibition was moderate with B-fraction. Similar results were obtained with dextran-induced edema.

Tests on the cardiovascular system in dogs and rats

In anesthetized dogs: B-fraction lowered the blood pressure of dogs in a graded manner

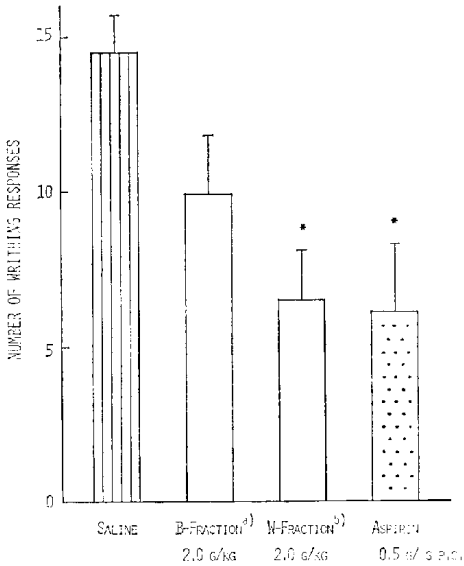


FIG. 2. Analgesic effect of mulberry root (Acetic acid method in mice).
 a) B-fraction: n-Butanol soluble fraction on mulberry root.
 b) W-fraction: Water soluble fraction of mulberry root.
 * Significant at P=0.01 vs control (saline). Vertical bars represent standard errors of means.

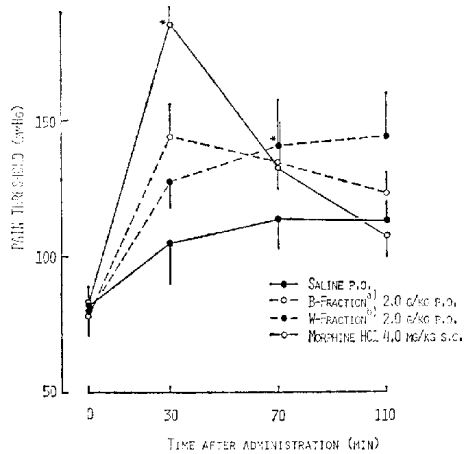


FIG. 3. Analgesic effect of mulberry root (Tail pressure method in rats).
 a) B-fraction: n-Butanol soluble fraction of mulberry root.
 b) W-fraction: Water soluble fraction of mulberry root.
 * Significant at P=0.01 vs control (saline). Vertical bars represent standard errors of means.

TABLE 2. Effect of mulberry root on carrageenin edema on the rat hind paw

Treatment	Dose g/kg, p.o.	No. of animals	Paw edema after the injection of carrageenin				
			1	2	3 (% ± S.E.)	4	5 (hr)
Saline		4	68.6 ± 17.5	123.5 ± 19.2	118.5 ± 15.1	108.1 ± 19.3	108.5 ± 19.7
B-fraction ^{a)}	1.0	4	41.8 ± 6.6	96.3 ± 6.2	94.1 ± 5.4	86.6 ± 7.1	67.0 ± 3.4
	3.0	4	44.5 ± 5.9	85.7 ± 1.3	85.1 ± 4.4	82.2 ± 5.0	72.2 ± 8.1
	5.0	4	50.0 ± 6.9	90.1 ± 16.1	83.3 ± 14.4	90.9 ± 11.7	86.1 ± 9.0
W-fraction ^{b)}	1.0	4	59.3 ± 2.3	91.8 ± 7.0	91.5 ± 3.0	84.9 ± 1.6	78.0 ± 3.9
	3.0	4	18.7 ± 4.0	47.4 ± 15.0 ^{c)}	65.1 ± 14.5 ^{c)}	69.7 ± 7.6	79.5 ± 5.0
	5.0	4	43.2 ± 6.3	56.0 ± 8.5 ^{c)}	81.5 ± 11.6	61.9 ± 4.1	73.3 ± 7.2

1% carrageenin 0.1 ml/rat paw s.c.

Drugs were orally administered 30 min before the injection of carrageenin.

a) B-fraction: n-Butanol soluble fraction of mulberry root.

b) W-fraction: Water soluble fraction of mulberry root.

c) Significant at P=0.05 vs Control (Saline).

within a dose range of 10 to 50 mg/kg i.v. The hypotensive response was biphasic and was accompanied by bradycardia following a transient reflex tachycardia (Fig. 4). These responses were slightly inhibited by atropine sulfate, 0.5 mg/kg i.v. On the other hand,

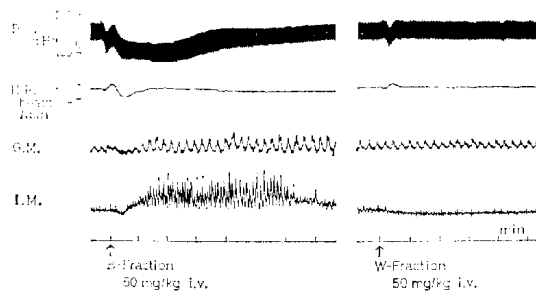


FIG. 4. Effects of mulberry root on blood pressure (B.P.), heart rate (H.R.), gastric (G.M.) and ileal movements (I.M.) in dogs. B-Fraction: n-Butanol soluble fraction of mulberry root. W-Fraction: Water soluble fraction of mulberry root.

W-fraction lowered blood pressure slightly. Gastric and ileal movements were significantly enhanced for about 10 min after the i.v. administration of B-fraction (50 mg/kg), but were not influenced by W-fraction.

Intraarterial injections of both fractions, in doses of 0.1 mg and above, increased brachial blood flow moderately. These vasodilating effects were significantly inhibited by 0.5 mg/kg i.v. of atropine.

In anesthetized rats: Like the results obtained in dogs, B-fraction produced a hypotensive effect accompanied with bradycardia in a graded manner within a dose range of 5 to 50 mg/kg i.v., while W-fraction caused a slight transient hypotensive effect. These responses to B-fraction were significantly inhibited by 0.5 mg/kg i.v. of atropine sulfate or by cervical bilateral vagotomy, but were not inhibited by 2 mg/kg i.v. of chlorpheniramine. Pretreatment of 50 mg/kg i.v. of each fraction did not alter the cardiovascular responses to acetylcholine, epinephrine, histamine, tyramine and cervical vagal stimulation in rats.

On the isolated rat atria: In the spontaneously contracting atria, B-fraction, 1 mg/ml, produced a marked increase in atrial rate and contraction following a slight decrease. On

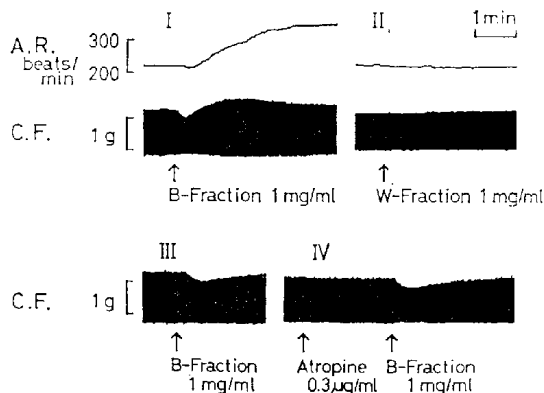


FIG. 5. Effects of mulberry root on the isolated rat atria. I and II: Spontaneously contracting atrium. III and IV: Electrically driven left atrium. C.F.: contracting force, and A.R.: atrial rate. B-Fraction: n-Butanol soluble fraction of mulberry root. W-Fraction: Water soluble fraction of mulberry root.

the other hand, W-fraction, 1 mg/ml, produced only a slight decrease or no response (Fig. 5, I and II). These responses to both fractions were dose-dependent. In the electrically driven left atria, B-fraction, 1 mg/ml, produced a significant decrease in contraction, which was not inhibited by the pretreatment of 0.3 μ g/ml of atropine sulfate (Fig. 5, III and IV), the concentration of atropine being sufficient to block the response to acetylcholine.

Tests on the isolated smooth muscle preparations (Guinea pig ileum, tenia caecum and vas deferens, and rat antrum and vas deferens)

B-fraction had a relaxant effect on the guinea pig ileum and an inhibitory effect on its spontaneous motility. These effects could be obtained with concentrations of 100 μ g/ml and over. B-fraction even in a concentration of 1 mg/ml did not prevent the spasmogenic effect of acetylcholine in concentrations of 10 and 30 ng/ml. In the guinea pig tenia caecum, B-fraction produced a significant relaxant effect. The maximum relaxation which paralleled that obtained by epinephrine (1 μ g/ml) was obtained by 300 μ g/ml of B-fraction. B-fraction had only a slight constricting effect on the rat antrum strip which is known to relax with β -adrenergic activity. In the guinea pig and rat vas deferens B-fraction caused no response, but it augmented the contractions produced by acetylcholine and epinephrine in the guinea pig vas deferens.

Diuretic activity in rats

Administration of both fractions in doses of 300 and 500 mg/kg given p.o. and i.p. increased slightly the urine volume as well as urinary excretion of Na and K ions.

Intestinal propulsion test in mice

Neither fraction in a dose of 2.0 g/kg p.o. produced an effect on intestinal propulsion.

Cathartic test in mice

As shown in Table 3, W-fraction in doses of 1 and 3 g/kg p.o. had a cathartic effect. The activity corresponded to that of 10% MgSO₄.

TABLE 3. Cathartic effect of mulberry root in mice

Treatment		Cathartic Effect			
		1st administration ^{b)}		2nd administration ^{c)}	
		No. of diarrhea mice/No. of mice used	Score ^{d)}	No. of diarrhea mice/No. of mice used	Score ^{d)}
Saline	0.1 ml/10 g, p.o. \times 2	0/5	0	0/5	0
W-fraction ^{a)}	1 g/kg, p.o. \times 2	1/8	0.06 \pm 0.02	3/8	0.19 \pm 0.09
	3 g/kg, p.o. \times 2	5/8	0.38 \pm 0.13	8/8	0.81 \pm 0.09
	5 g/kg, p.o. \times 2	6/8	0.63 \pm 0.16	8/8	1.00 \pm 0
10% MgSO ₄	0.1 ml/10 g, p.o. \times 2	4/5	0.60 \pm 0.19	5/5	0.90 \pm 0.10

a) W-fraction: Water soluble fraction of mulberry root.

b) Observation 3 hr after the 1st administration of a test substance.

c) Observation 3 hr after the 2nd administration of a test substance.

d) Scoring: Normal (0), Swelling (0.5), and Fluid-like feces (1.0).

Antitussive test in guinea pigs

Both fractions in doses of 300 and 500 mg/kg i.p. had a slight antitussive effect. The onset of action after administration of B-fraction was faster than that of W-fraction.

Corneal anesthesia in guinea pigs

No inhibition of corneal reflex was observed in 0.25 and 1.00% solutions of W-fraction 5 to 10 min after administration. Procaine hydrochloride in a concentration of 2% produced a complete disappearance of corneal reflex 5 to 10 min after administration.

DISCUSSION

Estimation of the pharmacological properties of both B- and W-fractions of mulberry root was attempted from the results of blind screening. Both fractions had common pharmacological effects with the exception of the cardiovascular system. The similarity of their pharmacological effects may be partly attributed to incompleteness of separating effective components into either fraction, since 20% of the water soluble part is not extracted by water but by n-butanol.

Since LD₅₀ of both fractions in different routes (i.v., i.p., p.o.) was more than 5 or 10 g/kg, the toxicity was therefore considered to be very low. From neuropharmacological observations, both fractions were estimated to produce CNS-depression such as sedative, tranquilizing, muscle relaxant, and analgesic activities as revealed by their inhibitory effects on spontaneous and exploratory movements, touch and pain responses, traction test, and grip and body tones.

Their analgesic and anticonvulsive activities were confirmed in detailed tests. Hypothermic effect of B-fraction was seen in mice with i.p. administration, but not in rats with the oral administration. This discrepancy may be attributed to the difference in dose, route or species. Anti-inflammatory activities of both fractions were also found in carrageenin- and dextran-induced edema tests.

B-fraction depressed the contractile force in the isolated electrically driven left atria of rats, while it augmented the contractile force as well as the atrial rate in the isolated spontaneously contracting atria of rats. Why responses in both atrial preparations differed remains to be investigated. The hypotensive, negative chronotropic and vasodilating activities in intact animals were partially blocked by the pretreatment of atropine or by bilateral cervical vagotomy, but the depressive effect on the isolated rat atria was not inhibited by atropine. The circulatory effects of B-fraction may thus be partly due to influences on the cholinergic innervation. Our results differ from those of Tanemura (9) who concluded that the actions of the root bark of mulberry tree are due to acetylcholine and its analogues presumably contained in the alcohol soluble fraction. We did not detect acetylcholine or its analogues in the B-fraction. B-fraction produced a significant relaxation in the isolated guinea pig ileum and tenia caecum, and no effect in the vas deferens, while acetylcholine contracted all the preparations.

Although the correlation between the clinical effects of Sohakuhi in Chinese medicine and the pharmacological effects have not been studied, we estimated a correlation based on

our own results. In noted technical books in Chinese medicine, "Wakansho" (1) and "Kampo Shoho Kaisetsu" (2), it was noted that Sohakuhi was clinically prescribed for catharsis, diuresis, alleviation of cough, edema and fever, and prevention of palsy. The cathartic, diuretic, antitussive and anti-local edema effects of the root bark of mulberry tree were confirmed in our experiments. The sedative (or tranquilizing) and hypotensive effects of the mulberry root found in our animal experiments herein may explain the prevention of palsy accompanying cerebral apoplexy. There is thus a general correlation between our results and the clinical effects of Sohakuhi.

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