Notes

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Effects of Crude Drugs on Congestive Edema

Johji Yamahara, Yuko Takagi, Tokunosuke Sawada,[1a] Hajime Fujimura,[1b] Kiyoharu Shirakawa, Masayuki Yoshikawa, and Isao Kitagawa[1c]

Kyoto College of Pharmacy,[1a] Gifu University School of Medicine,[1b] and Faculty of Pharmaceutical Sciences, Osaka University[1c]

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Screening tests of crude drugs was carried out using a model system of congestive edema in rats. Some crude drugs containing saponin, *i.e.*, Akebiae Caulis, Platycodi Radix, Polygalae Radix, and Mi-saponin(which resembles senega saponin in chemical structure) showed anti-edmatous and diuretic actions. Concomitant use of phenylbutazone with Mi-saponin resulted in an increase in urine volume and anti-edmatous action.

Keywords——biological active principles of crude drugs; anti-edematous action; diuretic action; saponin; Akebiae Caulis; Platycodi Radix; Polygalae Radix; *Madhuca longifolia* L. (Mi)

The term OKETSU is a concept characteristic of traditional Chinese medicine, and means an accumulation of "bad blood," presumably including congestive disease. Little work has been done on the crude drugs which have traditionally been used to treat OKETSU and as diuretics. We therefore carried out screening tests to investigate the effects of crude drugs using the experimental congestive edema produced in rats by Eisenburger *et al.*[2] as a model system. Aescin[2,3] which is the saponin mixture present in the seeds of *Aesculus hippocastanum* L. has already been reported to be effective on this and other inflammatory disease models, so in the present work the authors investigated the effect of the related saponin present in the seeds of *Madhuca longifolia* L.(Mi).[4]

Experimental

Crude drugs used as experimental materials were collected or purchased. Coarse materials were macerated in 50% methanol with cooling. The extracts were concentrated to dryness at 50° under reduced pressure. The extracts were stored in a desiccator, and were triturated with powdered acacia to form a suspension before use. The composition of Mi-saponin used was similar to that reported in a previous paper,[5] *i.e.*, the ratio of Mi-saponin A, B and C was 5 : 5 : 1. Mi-saponin was obtained in about 40% yield from the methanol extract of Mi. Furosemide (Hoechst), phenylbutazone (Fujisawa) and hydrocortisone (Nakarai) were employed as reference drugs.

Male Wistar rats weighing about 150 g were divided into groups of 8. Physiological saline solution, 5 ml/100 g of body weight, was given to each animal orally about 20 hr before the start of the experiment to produce a fasting state. On the next day, the jugular veins were ligated bilaterally to induce congestive edema under anesthesia with intraperitoneal thiopental sodium, 30 mg/kg. The drugs were dissolved in

1) Location: *a) Misasagi Yamashina-ku, Kyoto, 607, Japan; b) 40, Tsukasamachi, Gifu, 500, Japan; c) 133-1 Yamadakami, Suita, Osaka, 565, Japan.*
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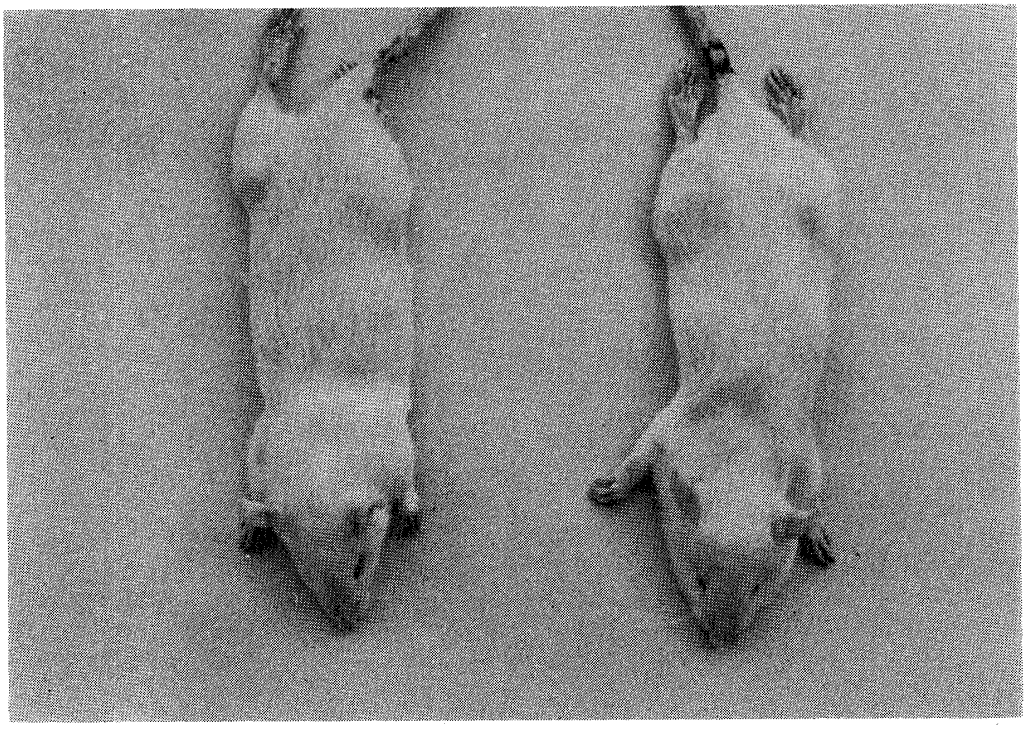

Fig. 1. Congestive Edema in Rats

right ---- operated control, left ---- sham-operated control.

physiological saline solution or suspended in acacia solution and given orally at a dose of 5 ml/100 g 3 hr after ligation. All animals were transferred to metabolic cages immediately after dosing and the 24 hr urine volume as well as urinary electrolytes including Na^+, K^+, and Cl^- and the severity of edema of the head (Fig. 1) were measured. Edema was measured by immersing the head to the level of the cervical vertebrae in water in a beaker with a side arm. The volume of water that overflowed was regarded as indicating the severity of congestive edema. The effect of each crude drug on congestive edema was expressed in term of its inhibition of edema.

Results

1. Results of Screening Tests

Table I shows the results. Moutan Cortex (cortex of *Paeonia moutan* Sims.) and Persicae Semen (seed of *Prunus persica* Batsch), which have been used as crude anti-OKETSU drugs, and Catalpae Fructus (fruit of *Catalpa ovata* G. Don), which is a diuretic drug, exerted no effect on urine volume or edema. Some crude drugs containing saponin, *i.e.*, Akebiae Caulis (stem of *Akebia quinata* Decne), Platycode Radix (root of *Platycodon grandiflorum* A. De Candolle) and Polygalae Radix (root of *Polygala tenuifolia* Willdenow) showed anti-edematous and diuretic actions. Sinomeni Caulis Et Rhizoma (stem and root of *Sinomenium actum* Rehd. *et* Wilson) and Cocculus Caulis Et Rhizoma (*Cocculus trilobus* D. C.) had anti-edematous action.

Mi-saponin, which resembles Senega saponin[6] (saponin of *Polygala senega* L. var. *latifolia* Torry *et* Gray), Platycodon saponin[7] and Polygala saponin[8] in chemical structure, had both anti-edematous and diuretic actions.

2. Anti-edematous and Diuretic Actions of Mi-saponin

As indicated in Table II, urine volume and edema were improved after the oral administration of Mi-saponin at a dose of 100 mg/kg as compared with those in the operated control

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Table I. Effects of Crude Drugs on Congestive Edema in Rats

Substance	Dose, mg/kg, *p.o.*	Urine volume ml/100 g, B.W.	Na^+mg	K^+mg mean±S.E.	Cl^- mg	Inhibition of Congestive edema (%)
Control (Sham operation)	—	2.5±0.02	19.4±1.1	23.1±2.0	25.9±2.4	—
Control (Operated)	—	1.3±0.04	9.1±0.8	24.6±2.1	12.2±1.0	0
Hydrocortisone	30	2.4±0.23	10.5±0.9	23.0±2.0	22.3±3.0	100**
Hydrocortisone	50	4.4±0.26*	15.8±2.1	24.0±2.1	24.2±1.0	100**
Furosemide	100	13.2±0.75***	27.9±3.1	26.8±2.2	52.0±2.8	100**
Aspirin	100	1.5±0.09	5.1±0.6	10.1±2.0	15.5±1.4	33
Polygalae Radix(Onji)[a]	2000	8.0±1.11**	10.2±1.1	26.2±1.9	14.2±1.5	100**
Senegae Radix (Senega)[b]	2000	4.0±0.40*	12.8±1.4	26.2±2.3	13.2±0.9	62*
Akebiae Caulis (Akebi)[b]	2000	4.1±0.23*	13.0±1.2	20.5±2.0	14.0±2.0	67*
Control (Sham operation)	—	2.2±0.12	20.5±1.8	24.5±2.0	25.8±2.4	—
Control (Operated)	—	1.2±0.05	8.8±0.3	21.5±1.8	10.5±1.0	0
Platycodi Radix (Kikyou)[b]	2000	3.1±0.51	10.1±1.0	39.2±2.9	20.1±1.7	100**
Mori Cortex (Souhakuhi)[a]	4000	1.5±0.05	6.7±0.4	19.0±1.3	13.4±1.2	53
Atractylodis Lanceae Rhizoma (Soujutsu)[a]	4000	1.6±0.24	8.9±0.5	15.9±1.7	13.3±1.2	34
Atractylodis Rhizoma (Byakujutsu)	4000	1.5±0.08	7.8±0.6	13.2±2.0	12.1±0.9	39
Alismatis Rhizoma (Takusha)	4000	1.6±0.11	8.8±1.0	20.1±2.0	10.1±0.5	36
Astrogali Radix (Ougi)[a]	4000	2.1±0.21	10.5±1.2	23.5±1.0	13.5±1.5	54
Gypsum Fibrosum (Setsuko)[b]	4000	2.6±0.23	14.1±1.1	20.1±2.0	15.2±1.7	46
Catalpae Fructus (Kisasage)[b]	4000	1.5±0.04	4.1±0.5	24.3±1.6	18.5±2.7	19
Plantagae Semen (Shazenshi)[a]	4000	1.8±0.09	5.7±0.5	24.3±1.6	15.7±1.9	11
Imperatae Rhizoma (Boukon)[b]	4000	1.5±0.02	9.4±0.1	23.1±2.0	14.1±1.1	0
Control (Sham operation)	—	2.4±0.02	18.9±2.0	28.1±2.0	26.8±2.1	—
Control (Operated)	—	1.1±0.22	8.0±0.6	20.1±1.1	10.0±1.7	0
Angelicae Radix (Touki)[b]	2000	2.2±0.06	10.1±0.3	20.1±1.1	12.1±2.0	10
Rhei Rhizoma (Daiou)[a]	2000	2.5±0.30	(Diarrhea)			
Nupharis Rhizoma (Senkotsu)[b]	4000	4.2±0.20*	18.4±1.5	35.2±2.0	41.8±2.1	76*
Moutan Cortex (Botanpi)[a]	2000	1.1±0.06	7.9±0.5	21.5±1.5	13.4±1.2	11
Persicae Semen (Tounin)[a]	2000	2.3±0.28	14.5±2.0	21.5±2.8	15.5±1.6	45
Myricae Cortex (Youbaihi)[b]	2000	2.6±0.12	7.8±0.5	18.9±1.1	10.8±1.0	55
Control (Sham operation)	—	2.5±0.40	20.1±0.5	30.0±2.1	24.3±1.8	—
Control (Operated)	—	1.1±0.74	9.2±0.5	25.1±2.0	10.0±1.4	0
Carthami Flos (Kouka)[b]	4000	1.5±0.02	4.1±0.4	20.1±2.0	15.4±1.6	36
Coptidis Rhizoma (Ouren)[a]	4000	1.9±0.32	9.5±0.8	26.4±1.7	18.4±1.1	12
Phellodendri Cortex (Oubaku)[a]	2000	1.5±0.12	4.1±0.3	17.2±1.3	10.5±1.0	5
Gardeniae Fructus (Sanshishi)[b]	4000	3.1±0.48	16.9±1.5	51.7±3.8	21.2±2.0	11
Control (Sham operation)	—	1.9±0.28	17.5±1.3	26.3±2.5	20.1±3.1	—
Control (Operated)	—	1.1±0.11	7.8±0.9	25.1±2.0	11.3±1.5	0
Angelica Decursivae Rhizoma (Zenko)[a]	2000	1.3±0.23	3.6±0.2	13.2±1.5	8.7±0.6	21
Coicis Semen (Yokuinin)[a]	2000	1.7±0.09	10.0±0.8	21.6±2.0	16.5±1.1	49
Sinomeni Caulis Et Rhizoma (Boui)[b]	2000	1.9±0.11	5.9±0.3	12.9±1.3	15.8±1.7	100**
Cocculus Caulis Et Rhizoma (Mokuboui)[b]	2000	2.6±0.32	7.8±0.3	18.5±1.4	10.3±1.5	91*
Trilobine	700	2.0±0.41	8.8±0.5	17.3±1.9	11.4±1.3	100**
Mi (Methanolic Ext.)	700	4.3±0.33*	15.1±1.4	30.1±4.0	20.3±2.4	100**
Mi-saponin	250	4.2±0.30*	16.7±1.1	27.8±2.1	20.5±2.5	100**

Significantly different from the control. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
a) Obtained from a market in Osaka.
b) Collected in the botanical garden of Kyoto College of Pharmacy.

Table II. Effect of Mi-saponin on Congestive Edema in Rats

Substance	Dose, mg/kg, *p.o.*	Urine volume ml/100 g, B.W.	Na^+mg	K^+mg mean±S.E.	Cl^- mg	Inhibition of congestive edema(%)
Control (Sham operation)	—	2.8±0.04	18.9±1.8	24.1±5.6	21.4±2.0	—
Control (Operated)	—	1.2±0.07	7.8±0.5	20.9±2.0	10.5±1.2	0
Furosemide	100	15.4±1.27***	30.5±2.8	28.1±2.0	36.9±3.3	100**
Mi (Methanolic Ext.)	350	2.5±0.15	14.5±1.0	20.4±1.8	16.3±1.0	60
Mi (Methanolic Ext.)	700	4.1±0.27*	23.5±1.9	25.5±1.4	22.5±4.0	100
Mi (Methanolic Ext.)	1000	5.5±0.31**	(Diarrhea)			
Mi-saponin	250	4.4±0.32*	15.9±1.4	24.4±3.3	18.7±1.3	100**
Mi-saponin	500	5.0±0.67*	17.8±1.2	23.5±2.2	19.0±2.9	100**
Mi-saponin A	250	4.0±0.45*	16.7±1.1	25.9±1.0	17.0±2.0	100**
Mi-saponin B	250	3.8±0.29*	15.9±2.3	24·9±0.8	15.9±2.1	100**

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

groups. The anti-edematous and diuretic actions of the methanolic extract of Mi were nearly proportional to the amount of saponin contained, and therefore active ingredients are presumably present in the saponin. The effects of Mi-saponin A and B were not significantly different from those of Mi-saponin.

3. Effect of Concomitant Use of Mi-saponin with Phenylbutazone

It is generally accepted that the side effect of phenylbutazone in man is edema due to sodium retention rather than gastrointestinal symptoms. Assuming that the side effects of phenylbutazone might be reduced by the diuretic action of Mi-saponin, we administered both drugs concurrently to rats (Table III), The decrease in edema was not marked after the administration of phenylbutazone alone at a dose of 200 mg/kg and the urine volume also remained unchanged. Concomitant use of phenylbutazone with Mi-saponin clearly increased the urine volume, and examination of urinary electrolytes revealed a marked increase in Na^+ excretion.

Table III. Effect of Mi-saponin in Combination with Phenylbutazone on Congestive Edema in Rats

Substance	Dose, mg/kg, *p.o.*	Urine volume ml/100 g, B.W.	Na^+mg	K^+mg mean±S.E.	Cl^- mg	Inhibition of congestive edema(%)
Control (Sham operation)	—	2.3±0.20	18.9±1.3	25.4±2.1	24.9±2.5	—
Control (Operated)	—	1.2±0.21	8.5±0.4	20.1±3.0	10.5±1.4	0
Furosemide	50	10.5±0.22***	27.3±2.1	27.6±2.0	48.9±3.3	100**
Furosemide	100	14.7±0.13***	29.8±1.9	31.7±2.7	53.0±4.4	100**
Phenylbutazone	25	1.9±0.03	10.7±1.1	23.6±2.0	15.7±1.4	16
Phenylbutazone	50	2.7±0.55	19.2±1.5	27.4±2.0	22.0±0.9	24
Phenylbutazone	100	2.4±0.10	8.3±0.7	21.8±1.9	14.1±1.1	16
Phenylbutazone	200	1.3±0.18	13.7±1.0	22.9±2.0	18.0±1.5	17
Mi-saponin	100	2.8±0.26	13.3±1.1	22.9±2.0	18.0±1.5	42
Mi-saponin	250	4.0±0.31*	13.4±2.0	28.2±2.8	16.7±2.4	100**
Phenylbutazone+Mi-saponin	25+100	3.5±0.13*	18.7±1.6	31.4±3.0	17.6±0.6	45
Phenylbutazone+Mi-saponin	50+100	5.4±0.29**	22.6±1.9	26.9±1.7	35.4±2.6	37
Phenylbutazone+Mi-saponin	100+100	4.4±0.52*	15.3±1.1	25.1±1.9	23.0±0.8	100**

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Discussion and Conclusion

We investigated the possible pharmacological activity of crude drugs that have been used traditionally to treat OKETSU, but failed to find any drugs having diuretic and anti-edematous actions. In contrast, crude drugs containing saponin showed anti-edematous and diuretic actions. Mi-saponin showed anti-edematous and diuretic actions that were dose-dependent, *i.e.*, essentially proportional to the amouht of saponin present in the crude drugs. Therefore, active ingredients were assumed to be present in the saponin. Concomitant use of phenylbutazone with Mi-saponin resulted in an increase in urine volume and anti-edematous action. Further study seems worthwhile. A previous study[9] failed to find anti-inflammatory and anti-edematous actions of crude drugs of Cocculus. However, an alkaloid of Cocculus, trilobine, showed apparent anti-edematous action on congestive edema in this work. Further studies on active ingredients of crude drugs which showed activity in the present experiments are in progress.

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Chemistry of Salicylic Acid and Anthranilic Acid. III.[1] Hypoglycemic Screening Tests for Salicylic and Anthranilic Acid Derivatives

Hiroyuki Asakawa, Eiko Imamiya and Yukihiko Hamuro

Central Research Division, Takeda Chemical Industries, Ltd.[2]

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The hypoglycemic activities of O-substituted salicylic acids (**1**—**11**), N-substituted anthranilic acids (**18**—**27**) and related compounds (**12**—**17**, **28**—**30**) were investigated. It was concluded that the presence of a chlorine atom *ortho* to the carboxy group in salicylic and anthranilic acid derivatives might enhance the hypoglycemic activity.

Keywords—— salicylic acid derivatives; thiosalicylic acid derivatives; anthranilic acid derivatives; chlorine atom *ortho* to carboxy group; hypoglycemic activity

Although salicylates have long been known to have hypoglycemic activity, their usefulness as hypoglycemic agents has been limited by the large doses required to bring about a significant lowering of blood glucose.[3,4] However, the effectiveness of sodium salicylate or acetylsalicylic acid in decreasing glycosuria and hyperglycemia in various animal preparations[3] at a dose level of about 500 mg/kg (*s.c.*) and in man[4] at about 5 g/day, is well established.

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