

Study of Various Rhubarbs Regarding the Cathartic Effect and Endotoxin-Induced Disseminated Intravascular Coagulation

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Sennoside A content in hot-water extracts from 17 varieties of rhubarb obtained from the market was measured, and their respective cathartic effects were also examined in mice. A positive correlation was confirmed between the sennoside A content and cathartic effects in our experiments. Furthermore, the effects of Shisendaio (四川大黄), with a higher sennoside A content, and Kinmondaio (錦紋大黄), with a lower sennoside A content, exhibited endotoxin-induced disseminated intravascular coagulation (DIC) which related closely to Oketsu symptoms, and these effects were examined in rats. Kinmondaio exhibited weak inhibition on both reducing erythrocyte deformability and prolonging euglobulin lysis time (ELT) in DIC rats.

From our results, it is possible to evaluate rhubarb's cathartic effect, one of its main drug effects, by examining the rhubarb's sennoside A content. However, it is difficult to estimate the cathartic effect according to the general market name of Rhubarb. No dramatic effect was found on the experimental models used for Oketsu symptoms such as endotoxin-induced DIC.

Keywords Rhubarb; sennoside A; cathartic effect; endotoxin-induced disseminated intravascular coagulation; erythrocyte deformability; euglobulin lysis time

Rhubarb varieties have been very commonly used as a purgative since the olden days of Western medicine. According to ancient Chinese herbal literature, rhubarb is stated to have been used as oral hemostatic agents for the treatment of hematemesis, melena and metrorrhagia, and also for pains of the legs and waist, which are considered to be caused by Oketsu symptoms related closely to disseminated intravascular coagulation (DIC).¹⁾ In present Chinese medicine, it is largely employed as a purgative and is also applied as a hemostatic agent. In "Wakanyakuko (和漢薬考),"²⁾ a book written in the Meiji era of Japan, it is recorded to have been prescribed simply as a hemostatic agent in folk medicine, besides being utilized in the system of Kampo medicine. In our literary search for the history of rhubarb imported from China to Japan, we knew that rhubarb of a soft quality, Gao (雅黄) and Bateidaio (馬蹄大黄), had been imported during the Meiji and Showa eras of Japan.³⁻⁹⁾ Regarding pharmacological studies of rhubarb, cathartic effects¹⁰⁻¹⁴⁾ have been mainly reported, with a decreasing effect on the urea and nitrogen levels in blood,¹⁵⁾ as well as an anti-herpes effect.¹⁶⁾ However, the effect of rhubarb against Oketsu symptoms has not been investigated.

This study was designed to clarify the correlation between the cathartic effect and sennoside A content of 17 varieties of rhubarb obtained from markets of Chengdu (成都) in China, Hong Kong and Osaka, respectively. According to ancient Chinese herbal medicine, it is described that rhubarb is also effective on Oketsu symptoms, in addition to having a cathartic effect. Oketsu is a symptom peculiar to the traditional Chinese system of medicine and is considered to be closely related to DIC. Further, the effect of some rhubarbs on endotoxin-induced DIC was also investigated.

MATERIALS AND METHODS

Materials Seventeen varieties of Chinese rhubarb,

shown in Table I, were used in this experiments. The hot water extracts employed in the present study were extracted as follows: First, we reduced the rhubarb to a powder with a hand mixer (Saneishoji Co., Ltd.). Tenfold (w/v) hot water ($90 \pm 5^\circ\text{C}$) was added to each sample of powder and the mixture was agitated for 15 min. The extract was obtained from freeze-drying. The sennoside A content in each extract was determined by HPLC¹⁷⁾ as shown in Table I. The following drug was also used in this study: endotoxin (*Escherichia coli* 055: B5, Difco).

Animals Male Kwl: Wistar strain rats (180–200 g) and male Kwl: ddY strain mice (18–20 g) were used. They were maintained in an air-conditioned room with light from 7 a.m. to 7 p.m. The room temperature (about 23°C) and humidity (about 60%) were controlled automatically. A laboratory pellet chow (Clea Japan, Inc.) and water were given freely.

Cathartic Test The cathartic test in the ddY strain mice was performed by the method of Tsurumi *et al.*¹⁸⁾ Test substances suspended in water were administered orally 1 h before the test. The activities of the test substances were expressed as the 50% cathartic dose (ED_{50} ; mg/kg) by the method of Litchfield-Wilcoxon.

Endotoxin-Induced DIC Experimental DIC was induced by a modification of the method of Schoendorf *et al.*¹⁹⁾ Test substances suspended in water were administered orally to the Wistar strain rats. Endotoxin (0.3 mg/kg) was injected intravenously 1 h after the dose of test substances. Blood samples were withdrawn from the abdominal vein into plastic syringes at 4 or 24 h after the injection of endotoxin, while the rats were anesthetized with pentobarbital (44.2 mg/kg). As an anticoagulant, 0.01 M sodium ethylenediaminetetraacetic acid (EDTA) was used for platelet counts, and a 1:9 volume of 3.8% sodium citrate was used for erythrocyte deformability and fibrinogen determination. Platelets were counted with an automatic blood cell counter. Fibrinogen was determined according to the method of Quick.²⁰⁾ Erythrocyte

TABLE I. Sennoside A Contents and Cathartic Activities of Various Rhubarbs

Sample No.	Commercial name	Market	Extract yield (%)	Sennoside A content (mg/extract g)	Cathartic activity (ED ₅₀ : mg/kg)
S-1	炮製馬蹄大黃	Chengdu (成都), China	33.8	18.8	68
S-2	青海錦紋大黃	Hong Kong	35.2	4.1	119
S-3	貴州產大黃	Osaka, Japan	32.2	16.6	133
S-4	等外雅黃	Hong Kong	25.9	15.0	139
S-5	四川大黃	Osaka, Japan	29.8	16.3	141
S-6	青海大黃	Osaka, Japan	29.0	15.0	148
S-7	陰干馬蹄大黃	Chengdu (成都), China	35.4	3.7	148
S-8	四川大黃	Osaka, Japan	30.8	8.2	150
S-9	四川大黃	Osaka, Japan	29.2	18.4	169
S-10	馬蹄大黃	Chengdu (成都), China	32.1	8.1	173
S-11	雅黃	Osaka, Japan	32.2	7.8	176
S-12	小塊錦紋大黃	Hong Kong	33.1	6.7	197
S-13	2等雅黃	Hong Kong	25.4	5.7	239
S-14	錦紋大黃	Osaka, Japan	35.1	0.5	246
S-15	甘肅錦紋大黃	Hong Kong	25.9	1.1	264
S-16	甘肅錦紋大黃	Hong Kong	30.2	0.4	360
S-17	1等雅黃	Hong Kong	24.1	2.4	486

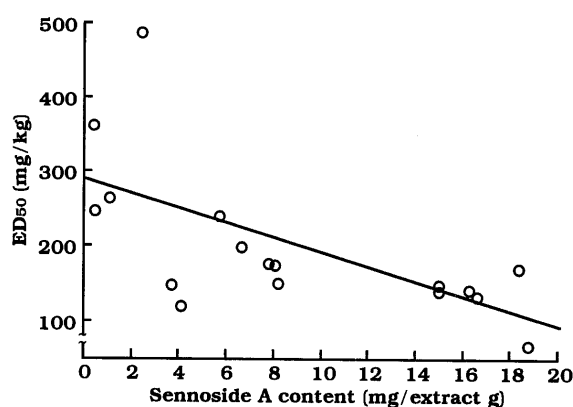


Fig. 1. Relationship between Sennoside A Content of Hot Water Extracts from Various Rhubarbs and Cathartic Activities

Sennoside A content; mg/extract g. Cathartic activity; ED₅₀ (mg/kg) in mice.
 $Y = -9.74X + 283.5$, $r = 0.639$.

deformability was expressed as erythrocyte filterability measured by the method of Reid *et al.*²¹⁾ Euglobulin lysis time (ELT) of the blood was measured by the method of Kaulla and Schultz.²²⁾

Statistical Analysis The experimental data were tested for statistically significant differences by means of Williams's Multiple Range test.

RESULTS

Content of Sennoside A and Cathartic Activity Sennoside A content and the cathartic activities of 17 varieties of rhubarb tested are shown in Table I. A weak correlation was recognized between the sennoside A content in the hot water extracts from rhubarb and the cathartic activity (Fig. 1).

Endotoxin-Induced DIC As shown in Fig. 2, the platelet count and fibrinogen level were decreased and erythrocyte deformability was reduced 4 h after the injection of endotoxin in the DIC rats, as compared to that of the intact rats. A weak inhibition by S-14 (50, 200 mg/kg) was observed against reducing erythrocyte

deformability, but it did not inhibit the decrease of platelet count and fibrinogen level. S-9 (50, 200 mg/kg) did not inhibit changes in these parameters in the DIC rats.

As shown in Fig. 3, the ELT was prolonged 24 h after the injection, as compared to that of the intact rats. S-14 (200 mg/kg) significantly inhibited the prolongation of ELT, but S-9 (50, 200 mg/kg) did not.

DISCUSSION

It is recognized that a correlation exists between sennoside A content, one of main active components in rhubarb, and cathartic activity. It is said that rhubarbs is also effective for improving Oketsu symptoms, as described in Chinese herbal literature, in addition to its cathartic effect. As shown in Fig. 1, a correlation between sennoside A content and cathartic effects was recognized. It has been generally claimed that the series of Gao (雅黃) and Bateidaio (馬蹄大黃), among rhubarbs in the market, possess a superior cathartic effect compared to other kinds of rhubarbs. However, sennoside A content did not necessarily show a higher value in those two series of rhubarbs. There is no written description of sennoside A content in rhubarb (Rhei Rhizoma) in Japanese Pharmacopoeia XII.

On the other hand, rhubarb has been also blended in prescriptions, such as "Daio-botampi-to (大黃牡丹皮湯)" and "Tokaku-jyoki-to (桃核承氣湯)" for treatment of Oketsu symptoms in the Chinese system and in the Kampo system of medicine. We reported that a number of crude drugs which were used to treat acute states of Oketsu symptoms exhibited some inhibitory effects on blood coagulation, thrombosis and fibrinolysis, while those used to treat chronic states of symptoms activated fibrinolysis and promoted erythrocyte deformability.²³⁻²⁸⁾ The effect of rhubarb against Oketsu symptoms was examined on the model of endotoxin-induced DIC. Among 17 varieties of rhubarb in which the sennoside A content was measured, S-9 (Shisendaio, 四川大黃), with a higher sennoside A content, and S-14 (Kinmondaio, 錦紋大黃), with a lower

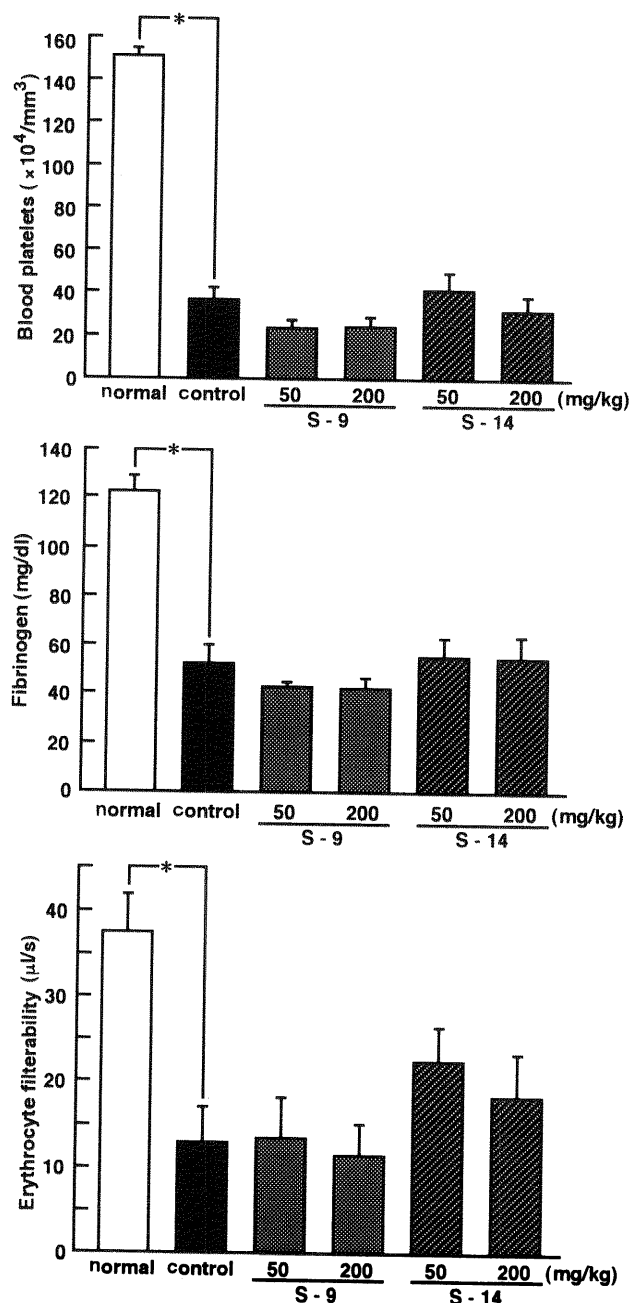


Fig. 2. Effects of Hot Water Extracts from Shisendaio (四川大黄, S-9) and Kinmondaio (錦紋大黄, S-14) on Blood Platelet Count, Fibrinogen Level and Erythrocyte Deformability in Endotoxin-Induced Disseminated Intravascular Coagulation (DIC) Rats

S-9 and S-14 were orally administered 1 h before the injection of endotoxin (0.3 mg/kg). Blood samples were collected from the abdominal vein 4 h after the injection of endotoxin and blood platelet count, fibrinogen level and erythrocyte deformability were determined. Each value represents the mean \pm S.E. of 13 rats. Significance, * $p < 0.01$.

sennoside A content, were supplied as samples for this examination. S-14 showed a tendency to inhibit the decrease of erythrocyte deformability at 4 h after intravenous injection of endotoxin, while it was not effective in decreasing the coagulative factor. S-14 also showed a significant inhibitory effect on the decrease of fibrinolytic activation which occurred after the intravenous injection of endotoxin.

Rhubarb varieties which are available in the market are different from original species containing hybrid species.

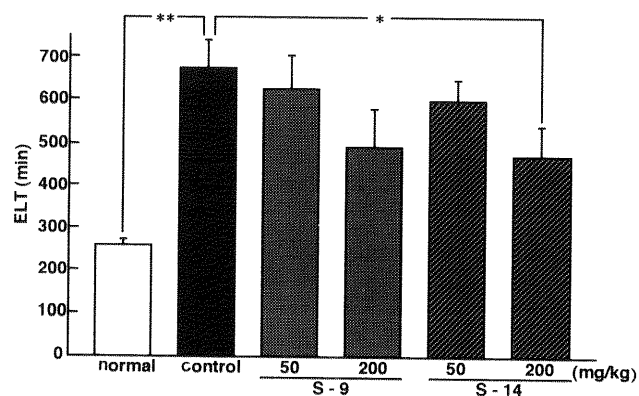


Fig. 3. Effects of Hot Water Extracts from Shisendaio (四川大黄, S-9) and Kinmondaio (錦紋大黄, S-14) on Euglobulin Lysis Time (ELT) in Endotoxin-Induced Disseminated Intravascular Coagulation (DIC) Rats

S-9 and S-14 were orally administered 1 h before the injection of endotoxin (0.3 mg/kg). Blood samples were collected from the abdominal vein 24 h after the injection of endotoxin. To the euglobulin fraction obtained from the blood was added thrombin, and ELT was determined by the time required for the coagulating euglobulin fraction to completely dissolve at 37°C. The control was orally administered water alone. Each value represents the mean \pm S.E. of 13 rats. Significance, * $p < 0.05$, ** $p < 0.01$.

The respective names were given to rhubarb according to the location of the original species and the shaped of the processed original rhizoma. The efficacy of various rhubarbs available in the market is difficult to predict on the basis of their respective names. Consequently, in the case when rhubarb would be used as a cathartic agent, we feel the sennoside A content should be at least measured. In the experimental models related to Oketsu symptom, rhubarbs were confirmed to exhibit weak improvement regarding erythrocyte deformability and weak fibrinolysis.

From these results, we think rhubarb should be used mainly for treatment of constipation as a cathartic agent. Further study is in progress on various models closely related to Oketsu symptom.

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