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Effect of Extract from Rhei Rhizoma on Adenine-Induced Renal Failure in Rats

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The effect of the extract from Rhei Rhizoma was examined in rats with renal failure induced by an adenine diet. On treatment with the rhubarb extract, the level of urea nitrogen and creatinine in the serum showed a significant decrease, indicating an improvement of renal function. The urea concentrations in the liver and kidney were also decreased after the treatment. In addition, administration of the rhubarb extract to rats markedly decreased the kidney weight and 2,8-dihydroxyadenine content in the kidneys. Macroscopic aspects of the kidneys, such as the appearance of the cut surface, were improved. However, there was no statistically significant difference between the control and rhubarb extract-treated groups with regard to the number of foreign body granulomas and deposits of acicular crystals (2,8-dihydroxyadenine).

Keywords——Rhei Rhizoma; renal failure; blood urea nitrogen; serum creatinine; liver urea; kidney urea; 2,8-dihydroxyadenine; morphological changes

Our previous reports showed that the extract from Rhei Rhizoma had an effect on urea nitrogen metabolism *in vivo* when administered to rats.[1-3] In particular, a single intraperitoneal administration of the extract caused a significant decrease of urea nitrogen in the serum. These experiments prompted us to examine whether the nutritional status of the animals modified the effect of rhubarb extract, especially in uremic rats. In the present study, the effect of rhubarb extract in an experimental animal model of renal failure induced by adenine feeding is described, with emphasis on the metabolic and morphological changes.

Materials and Methods

Animals and Diet——Male rats of the Wistar strain, initially weighing 90—100 g, were used in this experiment. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, type CE-2) for 2—3 d after arrival, then they were fed *ad libitum* on 18% casein diet containing 0.75% adenine for 6 to 30 d. The 18% casein diet had the following composition (in 100 g): casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[4] 4 g, vitamin mixture[4] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added at 0.75 g/100 g of diet. During the feeding period, the extract from Rhei Rhizoma (5 mg) was administered intraperitoneally to rats at 10 a.m. every day (rhubarb extract-treated group), while control rats were treated with an equal volume of saline (control group). The body weight and food intake of each rat were recorded every other day.

Extraction of Rhei Rhizoma——Roots of *Rheum officinale* BAILLON produced in China were finely powdered and extracted at 100 °C with water, as previously described.[1] The filtrate was concentrated under reduced pressure to obtain a brown residue.

Analysis——On the 6th, 12th, 18th, 24th, or 30th day of the feeding period, rats were sacrificed by means of a blow on the head and exsanguinated. Blood was collected in a conical centrifuge tube for the determination of urea nitrogen and creatinine. Urea nitrogen was determined by using a commercial reagent ("Urea NB-Test Wako" obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the urease–indophenol method and creatinine by a modification of the Folin–Wu method.[5]

Liver and kidneys were removed quickly, cooled on ice, and weighed rapidly. Portions of the liver and kidneys were homogenized with 9 volumes of ice-cold water in a Potter–Elvehjem type glass homogenizer. The homogenate, diluted about 100-fold with water, was used for the determination of urea by the method of Archibald.[6] For the histopathological studies, a portion of kidney was fixed in 10% buffered neutral formalin. Paraffin-embedded sections were stained with hematoxylin and eosin or other stains for the analysis of the renal lesion. Statistical significance of differences was determined by means of Student's t-test.

Results

Body Weight and Food Intake

Figure 1 compares the body weight of the rhubarb extract-treated and control rats fed on a 0.75% adenine diet. In the control group, the feeding of adenine diet caused a decrease of body weight until the 6th day. The body weight fell to 85% of that before adenine feeding, and then increased gradually from 6 to 30 d. In the rhubarb extract-treated group, the body weight was increased by about 5—10% over that of the control group. On the other hand, food intake (5.5—7.5 g/d/rat) of each group was almost proportional to the weight change throughout the experimental period.

Biochemical Findings

Table I shows the urea nitrogen and creatinine levels in the sera of rats of the rhubarb extract-treated and control groups. In the control group, administration of adenine to rats moderately increased the level of serum urea nitrogen. Furthermore, the level of creatinine showed a significant increase to 2.27 mg/100 ml on average at the end of the experiment,

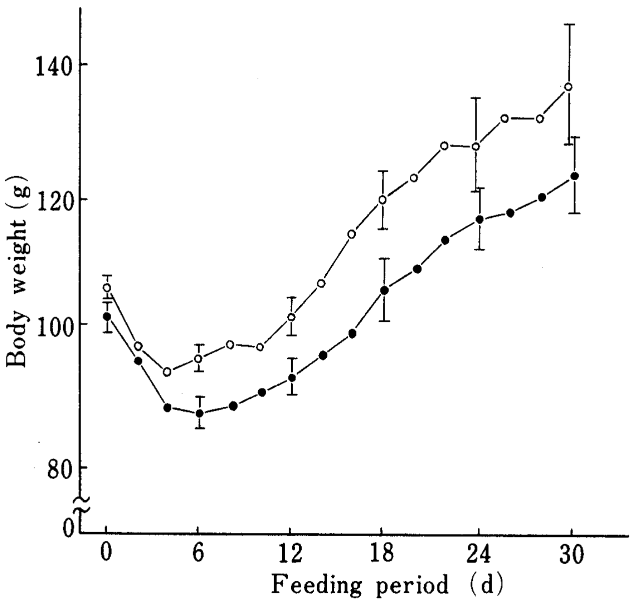


Fig. 1. Comparison of Body Weight of Rats of the Control and Rhubarb Extract-Treated Groups

●—●, control group; ○—○, rhubarb extract-treated group.

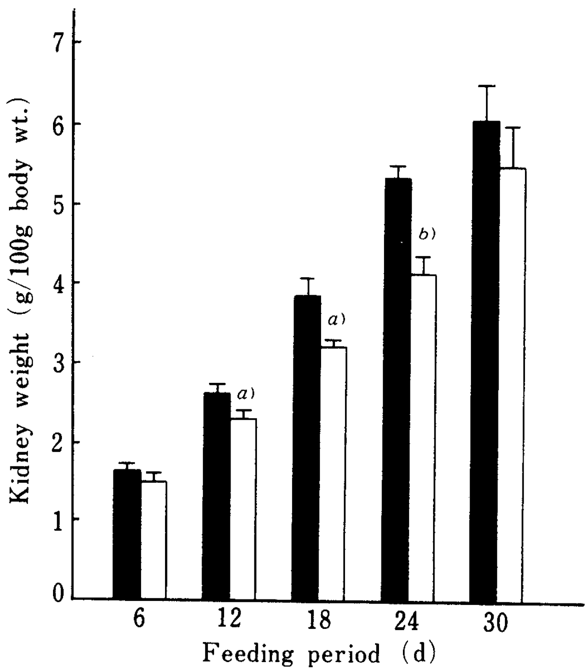


Fig. 2. Comparison of Relative Kidney Weight of Rats of the Control and Rhubarb Extract-Treated Groups

■, control group; □, rhubarb extract-treated group.

a) Significantly different from the control group, $p<0.05$. *b)* $p<0.01$.

TABLE I. Effect of Rhubarb Extract on Urea Nitrogen and Creatinine in Serum

Feeding period (d)	Group	Urea-N (mg/100 ml)		Creatinine (mg/100 ml)	
6	Control	28.3 ± 1.0	(100)	1.15 ± 0.01	(100)
	Rhubarb extract	$24.8 \pm 1.3^{a)}$	(88)	$1.01 \pm 0.02^{c)}$	(88)
12	Control	31.3 ± 1.7	(100)	1.33 ± 0.04	(100)
	Rhubarb extract	31.6 ± 1.3	(101)	$1.16 \pm 0.03^{b)}$	(87)
18	Control	30.9 ± 2.3	(100)	1.45 ± 0.04	(100)
	Rhubarb extract	$24.9 \pm 1.2^{a)}$	(81)	$1.19 \pm 0.04^{c)}$	(82)
24	Control	59.5 ± 4.5	(100)	1.70 ± 0.06	(100)
	Rhubarb extract	$38.7 \pm 4.5^{b)}$	(65)	$1.40 \pm 0.06^{b)}$	(82)
30	Control	67.4 ± 7.4	(100)	2.27 ± 0.10	(100)
	Rhubarb extract	73.4 ± 8.0	(109)	2.20 ± 0.13	(97)

Values are means $\pm$ S.E. of 6 rats.
Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$. *b*) $p<0.01$. *c*) $p<0.001$.

TABLE II. Effect of Rhubarb Extract on Urea Concentration in the Liver and Kidney

Feeding period (d)	Group	Liver urea (mg/g tissue)		Kidney urea (mg/g tissue)	
6	Control	0.40 ± 0.02	(100)	0.87 ± 0.05	(100)
	Rhubarb extract	$0.35 \pm 0.02^{a)}$	(88)	$0.69 \pm 0.04^{b)}$	(79)
12	Control	0.34 ± 0.02	(100)	0.44 ± 0.03	(100)
	Rhubarb extract	0.38 ± 0.01	(112)	0.46 ± 0.02	(105)
18	Control	0.40 ± 0.02	(100)	0.54 ± 0.03	(100)
	Rhubarb extract	$0.34 \pm 0.01^{a)}$	(85)	$0.44 \pm 0.01^{b)}$	(81)
24	Control	0.76 ± 0.05	(100)	0.86 ± 0.02	(100)
	Rhubarb extract	$0.48 \pm 0.05^{b)}$	(63)	$0.65 \pm 0.06^{b)}$	(76)
30	Control	0.87 ± 0.06	(100)	1.10 ± 0.07	(100)
	Rhubarb extract	0.84 ± 0.06	(97)	1.13 ± 0.09	(103)

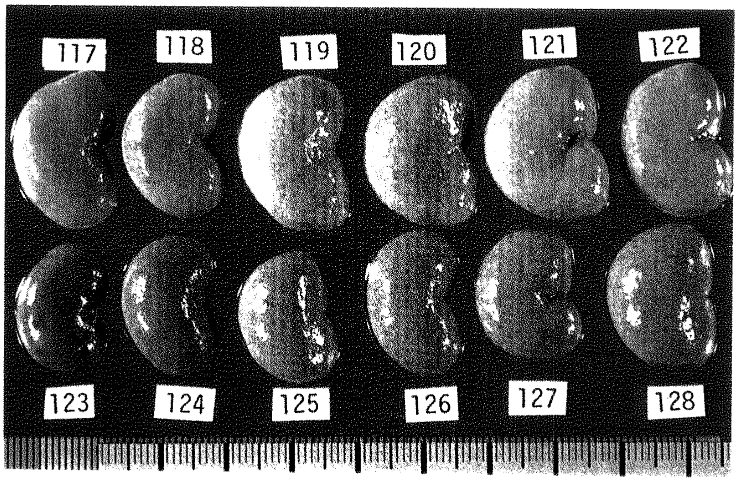
Values are means $\pm$ S.E. of 6 rats.
Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$. *b*) $p<0.01$.

indicating an impairment of renal function. Under these dietary conditions, the level of serum urea nitrogen was significantly lower at the 6th, 18th, and 24th days in rats of the rhubarb extract-treated group, but there was no statistically significant difference between the control and rhubarb extract-treated groups at the 12th and 30th days. In addition, serum creatinine level was decreased by 12—18% at the 6—24th days as compared with the control group (Table I).

On the other hand, the liver and kidney are known to be sites of ureapoiesis, since they contain all the enzymes of the urea cycle, and urea synthesis takes place in these organs.[7,8] Therefore, it seemed of interest to determine whether or not the intraperitoneal administration of rhubarb extract modified the urea concentrations in the liver and kidney. The results of this experiment are presented in Table II. On treatment with the rhubarb extract, the urea concentrations in the liver and kidney were significantly decreased by 12—37% at the 6th, 18th, and 24th days. In particular, the urea concentration showed a significant decrease at the 24th day, indicating improvement of the uremia.

Histopathological Findings

Figure 2 compares the relative kidney weight of the rhubarb extract-treated and control groups in rats fed on the adenine diet for 30 d. As shown in Fig. 2, the relative weight of the kidneys in the control group increased progressively from 1.79 g/100 g body weight at the 6th day to 6.06 g/100 g body weight at the 30th day. Macroscopically, the kidneys of rats fed on the adenine diet were markedly enlarged and pale brown in colour. In the cut surface, numerous scattered yellow-whitish grains were observed in the parenchyma, especially in the cortex (Fig. 3). Microscopically, numerous acicular crystals were seen, mainly in the tubular lumina, but rarely in the tubular epithelia and in the interstitium of the kidneys, and the formation of foreign body granuloma by marked reaction of the tubular epithelia, histiocytes, and foreign body giant cells against the acicular crystals was noted. Furthermore, direct analysis of the HCl extract of the crystals was performed according to the method of Bendich *et al.*[9] The preparation had ultraviolet (UV), infrared (IR) KBr, and mass [*m/z*: 167 (M^+, $C_5H_5N_5O_2$)] spectra similar to those of 2,8-dihydroxyadenine. From these results, it is



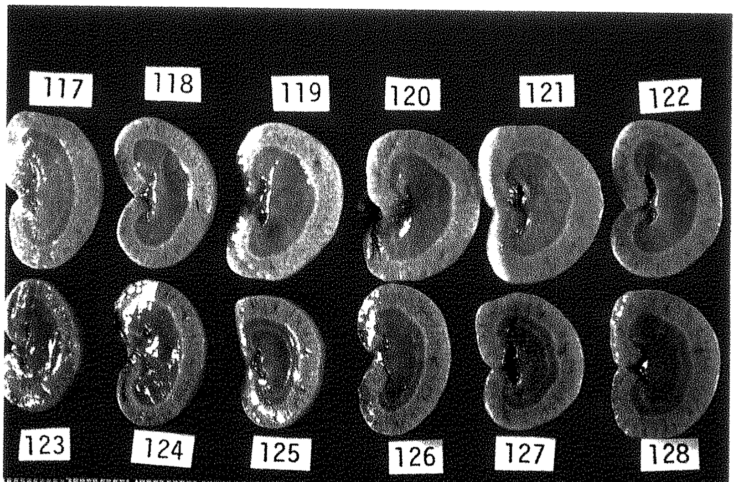


Fig. 3. Macroscopic Findings of the Kidneys in Rats of the Control and Rhubarb Extract-Treated Groups at the 24th Day

No. 117—122, control group; No. 123—128, rhubarb extract-treated group.

TABLE III. Effect of Rhubarb Extract on 2,8-Dihydroxyadenine Content in the Kidneys

Feeding period (d)	Group	2,8-Dihydroxyadenine (mg/kidneys)
6	Control	191 (100)
	Rhubarb extract	147 (77)
12	Control	433 (100)
	Rhubarb extract	396 (91)
18	Control	513 (100)
	Rhubarb extract	403 (79)
24	Control	690 (100)
	Rhubarb extract	494 (72)
30	Control	888 (100)
	Rhubarb extract	934 (105)

Six rats were used in each group.
Figures in parentheses are percentages of the control value.

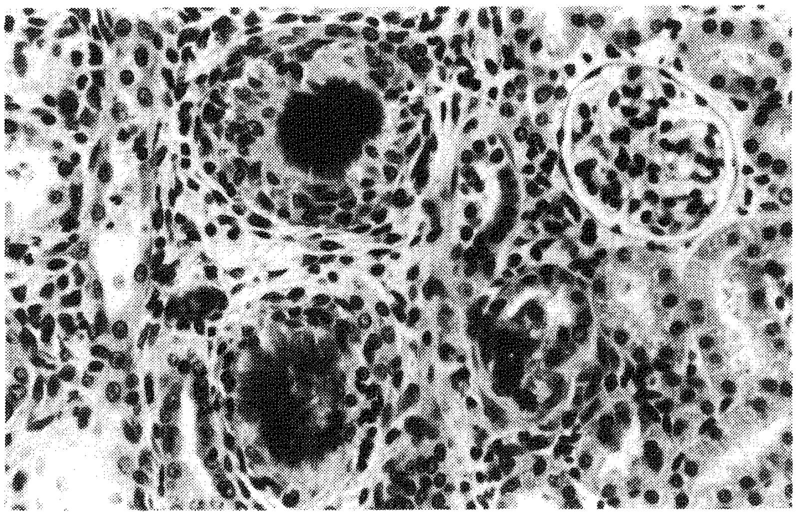


Fig. 4. Microscopic Findings of the Kidneys in Rats of the Rhubarb Extract-Treated Group at the 18th Day

Hematoxylin–eosin stain, ×300.

assumed that the administration of adenine may cause impairment of renal function by tubular obstruction with 2,8-dihydroxyadenine deposits. However, the rats of the rhubarb extract-treated group showed a moderate decrease of relative kidney weight; as shown in Fig. 2, the relative kidney weight was about 12—22% less at the 12th, 18th, and 24th days in the rhubarb extract-treated group as compared with the control group. In addition, the 2,8-dihydroxyadenine contents in the kidneys of rats in both groups were compared. As shown in Table III, a moderate decrease of 2,8-dihydroxyadenine content in the kidneys of the rhubarb extract-treated group was noticed at the 6th, 18th, and 24th days. Furthermore, macroscopic alterations of the kidneys were evident at the 12th, 18th, and 24th days, especially at the 24th day. Improvement of the appearance of the cut surface of the kidneys was observed (Fig. 3). However, microscopically there was no significant decrease in the number of foreign body granulomas against the acicular crystals (Fig. 4).

Discussion

Previous published data from our laboratory showed that the intake of adenine produced extraordinary increases of creatinine, urea nitrogen, and urea in the serum as well as a reduction in their urinary excretion.[10-12] Dietary adenine also caused a nephrotoxic condition as reflected in the morphological changes of the kidneys.[13-16] Macroscopically, the kidneys were markedly enlarged and pale brown in color.[12] The present data confirmed that the renal histological changes were characterized by degeneration of tubular epithelia with dilatation of the tubular lumina, deposits of amorphous birefringent crystals in the proximal and distal tubuli, and the formation of foreign body granuloma. These results show conclusively that rats fed an adenine diet are a useful experimental animal model of chronic renal failure. Disturbance of renal function induced by tubular obstruction by deposits of adenine or adenine metabolite can account for various biochemical features. From this point of view, the effect of rhubarb extract in rats with chronic renal failure induced by the adenine diet was examined in the present study.

As shown in Table I, the extract from Rhei Rhizoma caused a significant decrease in levels of both urea nitrogen and creatinine in the serum after repeated intraperitoneal administration in rats fed the adenine diet. In particular, the creatinine level was decreased by 12—18% at the 6—24th days as compared with the control value, indicating an improvement of renal function. These experimental results support the view that the extract from Rhei Rhizoma improves the renal clearance in the uremic state induced by adenine feeding.

Another important observation was that the administration of the rhubarb extract to rats reduced the increase in the kidney weight. As shown in Fig. 2, the relative weight of the kidneys was about 12—22% less at the 12th, 18th, and 24th days in the rhubarb extract-treated group as compared with the control group. This may be regarded as reflecting a decrease in the 2,8-dihydroxyadenine content in the kidneys (Table III). That is, a significant decrease of 2,8-dihydroxyadenine content in the kidneys was observed at the 6th, 18th, and 24th days. Thus, it may be considered that the prevention of 2,8-dihydroxyadenine production is the most important mechanism of action of rhubarb extract. Therefore, if possible, an appropriate dosage of rhubarb extract should be determined to control the 2,8-dihydroxyadenine deposition in the kidneys.

Morphologically, the kidneys in rats treated with the rhubarb extract showed a marked improvement in the macroscopic findings, such as the appearance of the cut surface of the kidney (Fig. 3). However, there was no statistically significant difference between the control and rhubarb extract-treated groups with regard to the number of foreign body granulomas and deposits of acicular crystals.

Although adenine metabolism in experimental animals is not completely understood, Clifford and Story[17] showed that dietary adenine increased the activities of hepatic adenine phosphoribosyltransferase (EC 2.4.2.7), 5′-nucleotidase (EC 3.1.3.5) and adenosine deaminase (EC 3.5.4.4), and the concentration of free adenine in the liver. Furthermore, Ho *et al.*[18] described the absorption and metabolism of orally administered purines. Adenine was incorporated into tissues to a greater extent than was guanine, hypoxanthine, or xanthine in both fed and fasted rats. Orally administered, radioisotope-labeled adenine was mostly excreted as allantoin. On the other hand, Bendich *et al.*[9] reported that adenine, especially when present at a supernormal concentration, is converted by xanthine oxidase to 2,8-dihydroxyadenine, a urate analogue characterized by an extremely low solubility. Although 2,8-dihydroxyadenine is considered to be a minor metabolic product in the breakdown of adenine, Philips *et al.*,[19] Shields *et al.*,[20] and Lindblad *et al.*[21] reported that its relative insolubility makes it a potential source of renal damage. Thus, adenine given orally in large doses may show nephrotoxicity attributable to 2,8-dihydroxyadenine. Allopurinol, a xanthine oxidase inhibitor, can prevent 2,8-dihydroxyadenine formation, but its effect may be considerably variable as indicated by Barratt *et al.*[22] However, it is of great interest that the nephrotoxic state caused by tubular obstruction with 2,8-dihydroxyadenine deposits can be mitigated by treatment with the rhubarb extract. The mechanism of the effect should be further studied in order to evaluate the possible therapeutic significance of the rhubarb extract, since many aspects, such as digestion, absorption, metabolism, and so forth, remain to be evaluated.

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