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Effect of extract from Salviae Miltiorrhizae Radix on the urinary urea, creatinine, and electrolyte excretion in uremic rats

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Abstract

The influence of aqueous extract from Salviae Miltiorrhizae Radix on the urinary excretion of urea, creatinine, and electrolyte was investigated in uremic rats induced by an adenine diet. The oral administration of Salviae Miltiorrhizae Radix extract resulted in a significant increment of urinary urea excretion. Furthermore, the excretion of urinary creatinine was remarkably increased in rats given Salviae Miltiorrhizae Radix extract throughout the experimental period. The level of urinary inorganic phosphate in rats which were administered Salviae Miltiorrhizae Radix extract was also increased significantly rather than in the control rats. There were, however, no significant differences in the excretion of urinary calcium and the urine volume in the two groups throughout the entire experiment. The improving action of uremic state by Salviae Miltiorrhizae Radix extract is discussed on the basis of the present results and the previously reported data [*J. Med. Pharm. Soc. for WAKAN-YAKU*, **2**, 446～451, 1985].

Key words Salviae Miltiorrhizae Radix, uremia, urea, creatinine, electrolyte, rat

Abbreviation Salviae Miltiorrhizae Radix (Tanzin) ; 丹参

Introduction

It was previously shown that the aqueous extract from Salviae Miltiorrhizae Radix was effective in uremic rats induced by an adenine diet, in the course of the screening of 15 crude drugs applied to the improvement of renal disease and hemostasis in traditional Chinese medicine.[1] The effect of Salviae Miltiorrhizae Radix on the uremic toxins was investigated in detail and as a result, it was found that the administration of aqueous extract from Salviae Miltiorrhizae Radix resulted in significant decreases of urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid, and inorganic phosphate and the marked increase of guanidinoacetic acid in the serum.[2] These results suggested that the aqueous extract from Salviae Miltiorrhizae Radix might improve the uremic state.

In the present paper, further studies were carried out researching for effect of aqueous extract from Salviae Miltiorrhizae Radix on the urinary excretion of urea, creatinine, and electrolytes in uremic rats induced by an adenine diet, in order to elucidate the possibility of whether Salviae Miltiorrhizae Radix is available to the uremia as a conservative agent.

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Materials and Methods

Animals and treatment : Male rats of the JCL : Wistar strain with a body weight of ca. 120 g were placed in metabolic cages under a temperature of 25 ± 1℃ and a 12 hrs dark-light rhythm. They were allowed an adaptation period of one week. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, type CE-2) during the adaptation period. Then they were fed *ad libitum* on an 18% casein diet containing 0.75% adenine for 24 days and the 48-hr urine sample collection was performed by 200 ml Erlenmeyer flask at 10 o'clock every other day. The 18% casein diet contained the following composition (in 100 g) : casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[3] 4 g, vitamin mixture[3] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. To this diet, adenine was added and mixed at the level of 0.75 g/100 g of diet. The procedure of an adenine diet produced uremic rats experimentally.[4-10] Throughout the experimental period, the aqueous extract from Salviae Miltiorrhizae Radix was orally administered to 8 rats in a dose of about 40 mg/rat/day which was reported to be the most effective previously,[2] whereas tap water was given to 6 rats.

Extraction of Salviae Miltiorrhizae Radix : The roots of Salviae Miltiorrhizae Radix (*Salvia Miltiorrhiza* BUNGE) produced in China, supplied by Tochimoto Tenkaido Co., Ltd., Osaka, Japan, were finely powdered and extracted with distilled water at 100℃ for 40 min (roots : water = 1 : 10, w/v), as described previously.[2] The aqueous extract was filtered with 4 layers of gauze and the filtrate was freeze-dried under reduced pressure and a brown residue was obtained in about 25% yield.

Analytical methods : Urea was determined by the method of Archibald.[11] Creatinine was assayed by the Folin-Wu method,[12] with a commercial reagent ("Creatinine-Test Wako") supplied by Wako Pure Chemical Industries, Ltd., Osaka, Japan. Calcium and inorganic phosphate were determined using the orthocresol -phthalein complex compound method[13] and molybdenum blue method[14] with commercial reagents which were "Calcium C-Test Wako" and "Phosphor B-Test Wako," respectively, purchased by Wako Pure Chemical Industries. All other assays were performed with reagent grade chemicals unless otherwise stated.

Statistics : Results were expressed as means ± S.E. with the Student's t-test being used for analysis of significance. A p value greater than 0.05 was considered not to be statistically significant.

Results

Effect of aqueous extract from Salviae Miltiorrhizae Radix on urine volume

The changes of the urine volume measured throughout the experimental period are presented in Fig. 1. In control rats fed on an adenine diet, the urinary volume was markedly increased to 50.5-68.0 ml/2 days on day 4-24. In contrast to this group, in rats fed on an 18 % casein diet, the urine volume was only about 28.0 ml/2 days reported previously[8] and an adenine diet produced polyuria. However, the urine volume in the rats treated with Salviae Miltiorrhizae Radix tended to rise a little higher in volume than in the control rats but the difference was not significant (Fig. 1). The total urine volume throughout the entire experiment was 626.7 ml/24 days in the control rats and 666.8 ml/24 days in Salviae Miltiorrhizae Radix-treated rats. In addition, there were no appreciable differences in the water intake of the two groups throughout the experimental period.

Effect of aqueous extract from Salviae Miltiorrhizae Radix on urinary urea excretion

Table I shows the effect of Salviae Miltiorrhizae Radix on the urinary urea excretion during the experimental period. The oral administration of Salviae Miltiorrhizae Radix extract resulted in a tendency to increase the urea output. The urinary urea was 188.0 ± 19.8, 263.6 ± 26.4, 246.8 ± 13.8, 216.6 ± 15.6, and 337.0 ± 44.0 mg/2 days in the Salviae Miltiorrhizae Radix-treated rats and 146.2 ± 11.2, 209.2 ± 19.0, 207.4 ± 22.4, 166.6 ± 25.4, and 252.2 ± 32.8

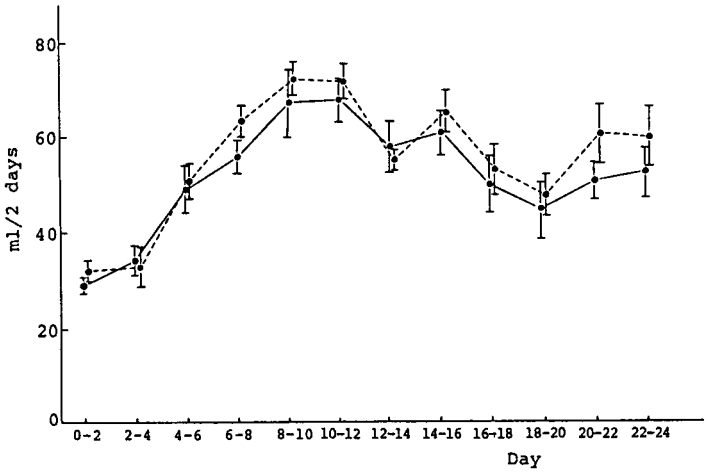

Fig. 1 Effect of extract from Salviae Miltiorrhizae Radix on urine volume. ●——●, control group ; ●·····●, Salviae Miltiorrhizae Radix extract-treated group.

Table I Effect of extract from Salviae Miltiorrhizae Radix on urea and creatinine levels in the urine.

	Day	Control		Salviae Miltiorrhizae Radix extract	
Urea (mg/2 days)	0 - 2	310.6±40.4	(100)	273.4±13.4	(88)
	2 - 4	352.6±20.4	(100)	321.0±37.4	(91)
	4 - 6	388.2±18.9	(100)	401.6±26.0	(103)
	6 - 8	298.4±99.2	(100)	381.8±34.8	(128)
	8 -10	146.2±11.2	(100)	188.0±19.8 [a)]	(129)
	10-12	291.0±30.6	(100)	303.4±19.6	(104)
	12-14	209.2±19.0	(100)	263.6±26.4 [a)]	(126)
	14-16	207.4±22.4	(100)	246.8±13.8 [a)]	(119)
	16-18	166.6±25.4	(100)	216.6±15.6 [b)]	(130)
	18-20	261.2±15.4	(100)	273.4±14.8	(105)
	20-22	252.2±32.8	(100)	337.0±44.0 [a)]	(134)
	22-24	298.0±48.8	(100)	351.8±20.4	(118)
Creatinine (mg/2 days)	0 - 2	7.06±0.30	(100)	8.08±0.18 [c)]	(114)
	2 - 4	5.18±1.10	(100)	6.16±0.78	(119)
	4 - 6	6.92±0.06	(100)	7.28±0.54	(105)
	6 - 8	5.86±0.50	(100)	7.94±0.74 [b)]	(134)
	8 -10	4.14±0.98	(100)	6.58±0.60 [b)]	(159)
	10-12	6.30±0.86	(100)	7.78±0.38 [a)]	(123)
	12-14	6.74±0.14	(100)	7.98±0.52 [b)]	(118)
	14-16	6.80±0.30	(100)	7.54±0.62	(111)
	16-18	5.42±0.58	(100)	8.10±0.40 [d)]	(149)
	18-20	6.20±0.64	(100)	8.46±0.58 [c)]	(136)
	20-22	4.74±0.74	(100)	7.46±0.76 [c)]	(157)
	22-24	5.34±0.64	(100)	8.50±0.48 [d)]	(159)

Values are means ± S.E. of 6 to 8 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, a) $p<0.20$, b) $p<0.10$, c) $p<0.05$, d) $p<0.01$.

mg/2 days in control rats on day 8-10, 12-14, 14-16, 16-18, and 20-22. The total urinary urea throughout the entire experiment was higher in the Salviae Miltiorrhizae Radix-treated than in control rats (3558.4 mg/24 days *vs.* 3181.6 mg/24 days, rise rate 12 %).

Effect of aqueous extract from Salviae Miltiorrhizae Radix on urinary creatinine excretion

As shown in Table I, the urinary creatinine was significantly higher in the rats given Salviae Miltiorrhizae Radix throughout the experimental period. The urinary creatinine level in the rats treated with Salviae Miltiorrhizae Radix rose to 7.46-8.50 mg/2 days compared with 4.74-7.06 mg/2 days in control rats on day 0-2, 16-18, 18-20, 20-22, and 22-24 and was higher significantly ($p<0.05$, $p<0.01$). The total urinary creatinine level in the Salviae Miltiorrhizae Radix-treated group throughout the experimental period also provided an appreciable increment by about 30 % as compared with the control group (91.9 mg/24 days *vs.* 70.7 mg/24 days).

Effect of aqueous extract from Salviae Miltiorrhizae Radix on urinary electrolyte excretion

Adenine-fed rats exhibited a significant increase in the urinary calcium, while the urinary excretion of inorganic phosphate showed a significant decrease during the feeding period as compared with the control diet rats. As shown in Table II, however, there were no significant changes of urinary calcium level in the Salviae Miltiorrhizae Radix-treated rats as compared with the control rats. Total urinary calcium throughout the experimental period was 3.30 mg/

Table II Effect of extract from Salviae Miltiorrhizae Radix on electrolyte levels in the urine.

	Day	Control	Salviae Miltiorrhizae Radix extract	
Ca (mg/2 days)	0 - 2	0.60±0.14 (100)	0.52±0.10	(87)
	2 - 4	0.38±0.04 (100)	0.34±0.08	(89)
	4 - 6	0.38±0.08 (100)	0.42±0.10	(111)
	6 - 8	0.32±0.12 (100)	0.34±0.06	(106)
	8 -10	0.38±0.10 (100)	0.26±0.08	(68)
	10-12	0.26±0.06 (100)	0.22±0.06	(85)
	12-14	0.14±0.02 (100)	0.16±0.02	(114)
	14-16	0.20±0.04 (100)	0.22±0.04	(110)
	16-18	0.20±0.02 (100)	0.18±0.02	(90)
	18-20	0.14±0.02 (100)	0.18±0.04	(129)
	20-22	0.14±0.02 (100)	0.16±0.04	(114)
	22-24	0.16±0.02 (100)	0.14±0.06	(88)
P (mg/2 days)	0 - 2	29.1±1.1 (100)	28.3±1.1	(97)
	2 - 4	28.2±1.0 (100)	31.9±2.5	(113)
	4 - 6	24.9±1.6 (100)	24.1±0.9	(97)
	6 - 8	19.6±0.8 (100)	23.5±1.5 [b]	(120)
	8 -10	22.9±0.8 (100)	24.9±0.8 [a]	(109)
	10-12	20.9±0.3 (100)	24.0±1.3 [b]	(115)
	12-14	17.9±0.9 (100)	20.7±0.9 [b]	(116)
	14-16	18.3±0.5 (100)	19.6±1.2	(108)
	16-18	16.7±0.5 (100)	16.9±0.4	(101)
	18-20	20.0±1.0 (100)	21.8±1.4	(109)
	20-22	21.7±1.1 (100)	22.2±0.6	(102)
	22-24	17.3±0.9 (100)	18.2±1.8	(105)

Values are means ± S.E. of 6 to 8 rats. Figures in parentheses are percentages of the control value. Significantly different from the control value, a) $p<0.20$, b) $p<0.10$.

24 days in the control rats and 3.14 mg/24 days in Salviae Miltiorrhizae Radix-treated rats, with little difference. On the contrary, the oral administration with Salviae Miltiorrhizae Radix resulted in the increase of urinary inorganic phosphate excretion by 9-20 % on the day 6-14. The total urinary inorganic phosphate was 257.5 mg/24 days and 276.1 mg/24 days in the control and the Salviae Miltiorrhizae Radix-treated group, respectively.

Discussion

In the uremic rats induced by an adenine diet, the effect of Salviae Miltiorrhizae Radix administered orally on the serum constituents was previously investigated and this crude drug resulted in the significant reduction of urea nitrogen with increasing dosage.[2] In the present study, the extract of Salviae Miltiorrhizae Radix markedly accelerated the excretion of urinary urea. From these results, it is conceivable that the decrease of blood urea nitrogen described previously[2] is due to increment of urinary urea output.

The level of urea in the body is used as a yardstick for the retention of compounds that may contribute to the toxicity of uremic state. Furthermore, blood urea nitrogen value has been shown to depend on the glomerular filtration.[15] From these considerations, the action of Salviae Miltiorrhizae Radix may be involved in the improvement of renal function.

On the other hand, Natelson and Sherwin suggested the theory that guanidinosuccinic acid might be formed from urea via the guanidine cycle.[16] Therefore, it may be conceivable that the decrease of guanidinosuccinic acid in serum described previously[2] is also attributed to the increment of urinary urea excretion.

On the contrary, it is well known that a given blood urea nitrogen value is influenced by protein diet. For these reasons, we need an indicator at least independent of protein intake. By combining serum with urine creatinine determination and calculating the creatinine clearance, one can assess the status of renal function with sufficient accuracy.[15] The decrease of creatinine level in serum which has been reported in the preceding paper[2] and the increment of urinary creatinine excretion in the present results are regarded as the rise of creatinine clearance by the extract from Salviae Miltiorrhizae Radix. It is conceivable that the lower creatinine level in serum is attributed to the increment of urinary creatinine excretion.

By the way, Cohen[17] and Mikami *et al.*[18] proposed that methylguanidine might be produced from creatinine. It thus seems plausible that the lower production of methylguanidine after administration with Salviae Miltiorrhizae Radix extract observed previously[2] is due to the rise of urinary creatinine excretion.

The oral administration of Salviae Miltiorrhizae Radix extract suggested the amelioration of uremic state by improving renal function which is represented by urea and creatinine. However, a mode of action of Salviae Miltiorrhizae Radix is presumably different from those of the rhubarb extract reported previously.[19-21] That is, the mechanism of uremia-improving action of rhubarb extract is considered to slow the progression of renal failure by developing the nitrogen-reutilizing system.

In uremic rats induced by an adenine diet, the serum calcium level was decreased, whilst the serum inorganic phosphate level was increased.[8] It is well known that the increase of serum inorganic phosphate is generally associated with a decrease of glomerular filtration rate.[22] In the preceding work, it has been reported that the Salviae Miltiorrhizae Radix extract decreases inorganic phosphate in the serum.[2] The urinary inorganic phosphate was increased in the present study. These results seem to be due to the increment of phosphate clearance.

Recently, it has been reported that the administration of Salviae Miltiorrhizae Radix has a beneficial effect which is manifested by a significant reduction in blood urea nitrogen and serum creatinine, and rise in creatinine clearance clinically,[23] which is thought to support the present results.

From the above observations, it is proposed that Salviae Miltiorrhizae Radix has a certain beneficial effect on improving uremic state which is induced as a result of hyperfiltration in remnant nephrons still functioning. Further studies on the mode of action and active principles of Salviae Miltiorrhizae Radix are currently in progress in our laboratory.

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