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Uremia-improving effect of Salviae Miltiorrhizae Radix in rats

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Abstract

An investigation was conducted on the influence of orally administered Salviae Miltiorrhizae Radix extract in rats with chronic renal failure induced by an adenine diet for 24 days. The rats treated with the extract from Salviae Miltiorrhizae Radix showed significant decreases in urea nitrogen and creatinine in the serum, indicating an improvement of hyperazotemia. The level of phosphate in the serum was also decreased after the treatment. Furthermore, a significant decrease in guanidino compounds, methylguanidine and guanidinosuccinic acid, was observed in the serum, while a marked increase in the level of guanidinoacetic acid was exhibited. Methylguanidine was not detectable in the serum of the Salviae Miltiorrhizae Radix extract-treated group given 20 and 40 mg/rat/day. Treatment of chronically uremic rats with the extract from Salviae Miltiorrhizae Radix resulted in an increase of serum glycine, serine, glutamic acid, aspartic acid, and isoleucine.

Keywords *Salvia miltiorrhiza* BUNGE, chronic renal failure, hyperazotemia, methylguanidine, guanidinosuccinic acid, guanidinoacetic acid, free amino acids

Abbreviations Aconiti Japonici Tuber (Bushi)；附子, Angelicae Radix (Tôki)；当帰, Cnidii Rhizoma (Senkyu)；川芎, Codonopsitis Radix (Tôjin)；党参, Ginseng Radix (Ninzin)；人参, Glycyrrhizae Radix (Kanzô)；甘草, Gorei-san (Wu-Ling-San)；五苓散, Hatimi-ziô-gan (Ba-Wei-Di-Huang-Wan)；八味地黄丸, Hoelen (Bukuryo)；茯苓, Onpi-tô (Wen-Pi-Tang)；温脾湯, Polyporus (Tyorei)；猪苓, Rhei Rhizoma (Daio)；大黄, Salviae Miltiorrhizae Radix (Tanzin)；丹参, San'ô-syasin-tô (San-Huang-Xie-Xin-Tang)；三黄瀉心湯, Sinbu-tô (Zhen-Wu-Tang)；真武湯, Syô-saiko-tô (Xiao-Chai-Hu-Tang)；小柴胡湯, Tyorei-tô (Zhu-Ling-Tang)；猪苓湯, Zingiberis Rhizoma (Kankyô)；乾姜

Introduction

A screening test for influences on renal failure in rats was performed on a total of 15 kinds of selected crude drugs, *i.e.*, main constitutive crude drug excluding Polyporus (*Polyporus umbellatus* FRIES) and Hoelen (*Poria cocos* WOLF) of Hatimi-ziô-gan, Tyorei-tô, Sinbu-tô, Gorei-san, Syô-saiko-tô, and San'ô-syasin-tô so far used for the improvement of renal diseases and hemostasis, and Cnidii Rhizoma (*Cnidium officinale* MAKINO), Angelicae Radix (*Angelica acutiloba* KITAGAWA), Codonopsitis Radix (*Codonopsis tangshen* OLIV. tangshen Oliver), and Salviae Miltiorrhizae Radix (*Salvia miltiorrhiza* BUNGE) reported to have an improving effect on uremia in renal insufficiency. As a result, some information suggestive of the uremia-improving effect of Salviae Miltiorrhizae Radix was obtained.[1] Therefore, in the present research, further studies were made in detail of Salviae Miltiorrhizae Radix's effect.

Materials and Methods

Animals and treatment：Male rats of the

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JCL : Wistar strain, initially weighing 110-120 g, were used in this experiment. The animals were fed on commercial feed (CLEA Japan Inc., Tokyo, type CE-2) for 2-3 days after arrival. Then they were fed *ad libitum* on an 18 % casein diet containing 0.75 % adenine for 24 days. 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[2] 4 g, vitamin mixture[2] 1 g, cellulose powder 2 g, and choline chloride 0.1 g. The procedure of adenine feeding produced experimental chronic renal failure.[3-9] During the adenine feeding period, the extract from Salviae Miltiorrhizae Radix was administered orally to rats as drinking water, while control rats received tap water. Throughout the experimental period, there was no statistically significant difference between the control and Salviae Miltiorrhizae Radix extract-treated rats with regard to body weight. Food intake of each rat was essentially proportional to weight change. On the 24th 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 and the serum was separated by centrifugation immediately after collecting the blood.

Extraction of Salviae Miltiorrhizae Radix : Roots of *Salvia miltiorrhiza* BUNGE produced in China, purchased from Tochimoto Tenkaidô Co., Ltd., Osaka, Japan, were made into a fine powder and extracted for 40 min at 100℃. The filtrate was lyophilized to produce a brown residue and was obtained in 25 % yield.

Analyses : All reagents were commercial products of the highest grade available. 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.[10] Creatinine was determined by using a commercial reagent (Creatinine - Test Wako) based on the Folin-Wu method.[11] Calcium was determined by using a commercial reagent (Calcium C-Test Wako) based on the orthocresol-phthaleic complex compound method.[12] Inorganic phosphate was determined by using a commercial reagent (Phosphor B-Test Wako) based on the molybdenum blue method.[13] For the determination of guanidino compounds, serum was deproteinized by the addition of trichloroacetic acid (TCA) (final concentration, 10 %). The supernatant obtained by centrifugation at 3000 rpm for 10 min was applied to a Shimadzu LC-5A liquid chromatograph using a stepgradient. A fluorescence spectrometer, model RF-540 (excitation 395 nm, emission 500 nm ; Shimadzu Co.) was used to monitor the effluent from the column. Free amino acids were determined with a Hitachi 835 high-speed amino acid analyzer. Before the determination, the serum was deproteinized by adding 3 volumes of 3 % sulfosalicylic acid.

Statistics : The significance of differences between the control and Salviae Miltiorrhizae Radix extract-treated groups was tested by the use of Student's *t*-test.

Results

Effect of extract from Salviae Miltiorrhizae Radix on urea nitrogen and creatinine levels in the serum

Table I shows the effect of the Salviae Miltiorrhizae Radix extract-treated and control groups. The extract from Salviae Miltiorrhizae Radix significantly reduced the urea nitrogen level by 31-33 % at 20 and 40 mg/rat/day as compared with the control, while the oral administration of 10 and 80 mg/rat/day showed no effect. The creatinine level was also decreased by 23-28 % as compared with the control upon oral administration of 20 and 40 mg/rat/day.

Effect of extract from Salviae Miltiorrhizae Radix on calcium and phosphate levels in the serum

The rats of the Salviae Miltiorrhizae Radix extract-treated group showed a moderate decrease of phosphate in the serum ; as shown in Table II, the value for serum phosphate was about 13-20 % lower at the dosage level of 10-80 mg/rat/day as compared with the control group. The level of serum phosphate showed a significant decrease at the 80 mg/rat/day, indicating improvement of hyperphosphatemia. In contrast, administration of the Salviae Miltiorrhizae Radix

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

Dose (mg/rat/day)	Urea nitrogen (mg/dl)		Creatinine (mg/dl)	
Control	123.1±16.1	(100)	2.35±0.15	(100)
10	92.6±11.1	(75)	2.05±0.21	(87)
20	84.8± 3.6[a)]	(69)	1.81±0.04[b)]	(77)
40	82.0± 4.4[a)]	(67)	1.69±0.06[b)]	(72)
80	93.0± 6.6	(76)	1.99±0.09	(85)

Values are means ± 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)]$p<0.01$

Table II Effect of extract from Salviae Miltiorrhizae Radix on calcium and phosphate levels in the serum.

Dose (mg/rat/day)	Ca (mg/dl)		P (mg/dl)	
Control	5.93±0.39	(100)	19.10±1.33	(100)
10	6.42±0.39	(108)	16.09±1.15	(84)
20	6.40±0.26	(108)	16.59±0.45	(87)
40	6.12±0.15	(103)	15.20±0.62	(80)
80	6.58±0.17	(111)	16.04±0.48[a)]	(84)

Values are means ± S.E. of 6 rats. Figures in parentheses are percentages of the control value. [a)]Significantly different from the control value, $p<0.05$.

Table III Effect of extract from Salviae Miltiorrhizae Radix on levels of guanidino compounds in the serum.

Dose (mg/rat/day)	MG (μg/dl)	GSA (μg/dl)	GAA (μg/dl)
Control	6.89±0.85 (100)	54.08±2.97 (100)	39.05± 8.85 (100)
10	2.51±1.59[a)] (36)	40.84±4.73[a)] (76)	44.49± 9.18 (114)
20	N.D.	37.35±2.04[c)] (69)	74.35±10.97[a)] (190)
40	N.D.	44.69±2.60[a)] (83)	74.21±13.53[a)] (190)
80	2.42±1.12[b)] (35)	56.54±6.53 (105)	92.88±12.21[b)] (238)

MG, methylguanidine ; GSA, guanidinosuccinic acid ; GAA, guanidinoacetic acid. Values are means ± 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)]$p<0.001$. N.D., not detectable.

extract had a lesser effect on the level of serum calcium. As shown in Table II, an increase (8-11 % compared to the control) in the calcium level was observed at the 10, 20, and 80 mg/rat/day level but was not statistically significant.

Effect of extract from Salviae Miltiorrhizae Radix on guanidino compounds in the serum

The concentrations of various guanidino

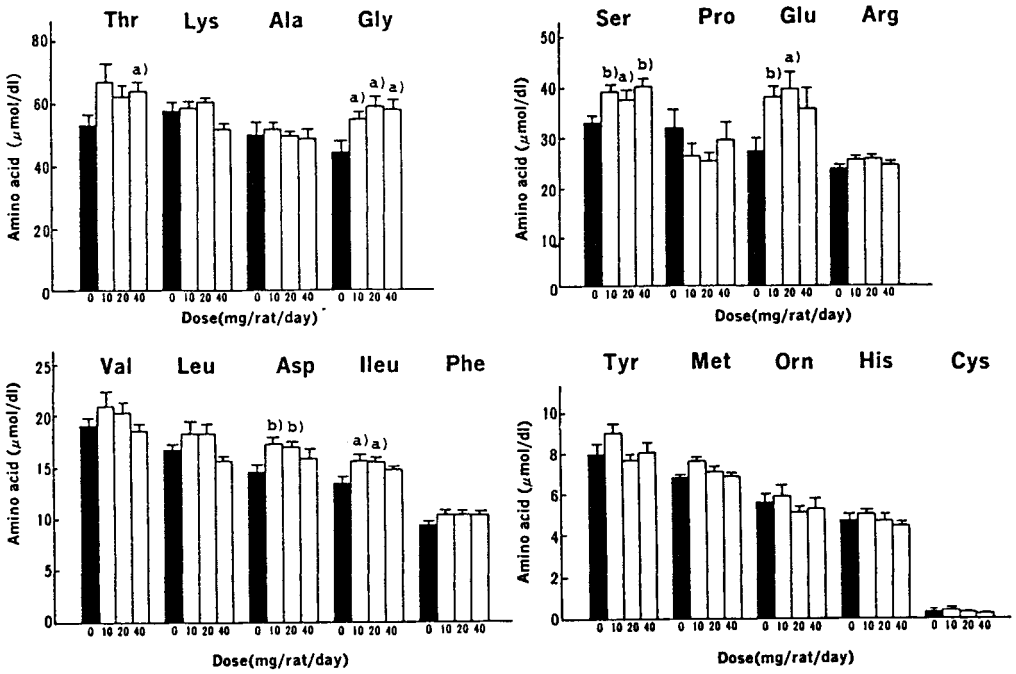

Fig. 1 Effect of extract from Salviae Miltiorrhizae Radix on free amino acids in the serum. Values are means ± S.E. of 6 rats. [a]Significantly different from the control value, $p<0.05$, [b]$p<0.01$.

compounds are shown in Table III. Administration of the Salviae Miltiorrhizae Radix extract to rats resulted in a decrease of guanidino compounds in the serum. As shown in Table III, the methylguanidine (MG) level in the serum of the Salviae Miltiorrhizae Radix extract treated-group was sharply decreased at the 10 mg/rat/day and was not detectable at 20 and 40 mg/rat/day. A decrease was also observed in the level of serum guanidinosuccinic acid (GSA) at the 10, 20, and 40 mg/rat/day dose level. The level of GSA was decreased to 37.35 μg/dl on average at 20 mg/rat/day. However, there was no statistically significant difference between the control and Salviae Miltiorrhizae Radix extract-treated groups at the 80 mg/rat/day dose level. On the other hand, the administration of 20, 40, and 80 mg/rat/day of the Salviae Miltiorrhizae Radix extract caused a significant increase of about 90-138 % in guanidinoacetic acid (GAA) level.

Effect of extract from Salviae Miltiorrhizae Radix on free amino acids in the serum

In an examination of the effects of oral administration of the extract from Salviae Miltiorrhizae Radix, it was found that the level of free amino acids in the serum was significantly increased. As shown in Fig. 1, Salviae Miltiorrhizae Radix extract induced an increase of serum glycine and serine levels at doses of 10 to 40 mg/rat/day. The glutamic acid, aspartic acid, and isoleucine levels were also increased at doses of 10 and 20 mg/rat/day. However, further increase in the dose to 40 mg/rat/day did not produce any further increase in these amino acid levels.

Discussion

Hyperazotemia, accumulation of uremic toxins, metabolic imbalance of amino acids and electrolytes, hypoalimentary condition, hormonal imbalance, *etc.* in serum were induced in rats by long term feeding of adenine and they bore close resemblance to metabolic abnormalities noted in chronic renal failure in humans.[3-9]

Previously, we made clear that Rhei Rhizoma and Onpi-tô (Rhei Rhizoma, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma, and Aconiti Japonici Tuber) remarkably lowered serum urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid and the like called uremic toxin,[14-17] and reported that Ginseng Radix and Aconiti Japonici Tuber, main constitutive crude drug of Onpi-tô, significantly lowered creatinine, methylguanidine and guanidinosuccinic acid, and guanidinosuccinic acid, respectively, in rats with adenine-induced chronic renal failure.[18] Subsequent screening of the above 15 kinds of crude drugs used so far for the improvement of renal disease and hemostasis led to some important information suggestive of Salviae Miltiorrhizae Radix's improving effect on uremia in rats.[1] A detailed examination made this time of Salviae Miltiorrhizae Radix's effects revealed its improving effects on uremic symptoms which can be observed with Rhei Rhizoma[14,15] and Onpi-tô[16,17] such as significant decreases in serum urea nitrogen, creatinine, methylguanidine, and guanidinosuccinic acid, tendencies to improving hyperphosphatemia, pattern of free amino acids in blood. In addition, an increase was observed in the level of serum guanidinoacetic acid level unlike the crude drug described above.

Further studies remain to be made of the action mechanism with which uremic symptoms can be improved by the extract from Salviae Miltiorrhizae Radix. As is obvious from the results of the present experiment, Salviae Miltiorrhizae Radix remarkably increased guanidino acetic acid level in blood. Guanidinoacetic acid, which is formed by transamidinase with arginine and glycine as substrate, is thought to be produced mostly in kidneys, and the formation of guanidinoacetic acid is recognized to decrease as renal dysfunction progresses.[19,20] From this it can be thought that unlike Rhei Rhizoma,[14,15] Onpi-tô,[16,17] Ginseng Radix,[18] and Aconiti Japonici Tuber[18] which have already been reported, Salviae Miltiorrhizae Radix has an action which partially repairs renal dysfunction, in view of interesting information that Salviae Miltiorrhizae Radix accelerates the elimination of urea and creatinine into urine.[21]

Salviae Miltiorrhizae Radix, whose pharmacological actions have so far been described as vasodilatation, hypotensive activity, antibacterial activity and so on in the text, recently began to be used in China for the elimination of pains due to coronary insufficiency and hemostasis and for the acceleration of vasodilatation.[22] There are also reported cases in which Salviae Miltiorrhizae Radix injection for emergency treatment of myocardial infarction resulted in rapid improvement of myocardial ischemic findings and in lowering of blood lipid.[22] On the other hand, Zhang *et al.*[23] recently reported that an intravenous drip of Salviae Miltiorrhizae Radix for the uremic therapy of chronic renal failure patients gave rise to remarkably effective results in 8 of 53 cases and effective results in 27, in terms of blood urea nitrogen, serum creatinine, and creatinine clearance. These clinical findings are thought to be experimentally supported in part by the uremia improving effect of Salviae Miltiorrhizae Radix which we observed in the present experiment.

References

1) Oura, H., Yokozawa, T. and Chung, H.Y. : Effect of crude drugs on serum constituents in rats with chronic renal failure. *J. Med. Pharm. Soc. for WAKAN-YAKU* **2**, 434-438, 1985

2) Harper, A.E. : Amino acid balance and imbalance. Part I. Dietary level of protein and amino acid imbalance. *J. Nutr.* **68**, 405-424, 1959

3) Yokozawa, T., Oura, H., Nakagawa, H. and Okada, T. : Adenine-induced hyperuricemia and renal damage in rats. *Nippon Nôgeikagaku Kaishi* **56**, 655-663, 1982

4) Yokozawa, T., Oura, H. and Okada, T. : Metabolic effects of dietary purine in rats. *J. Nutr. Sci. Vitaminol.* **28**, 519-526, 1982

5) Yokozawa, T., Oura, H., Zheng, P.D., Fukase, M., Koizumi, F. and Kanaoka, M. : Metabolic effects of dietary purine and pyrimidine bases in rats. *Agric. Biol. Chem.* **47**, 1297-1304, 1983

6) Yokozawa, T., Zheng, P.D. and Oura, H. : Experimental renal failure rats induced by adenine.—Evaluation of free amino acid, ammonia nitrogen and guanidino compound levels—. *Agric. Biol. Chem.* **47**, 2341-2348, 1983

7) Yokozawa, T., Zheng, P.D. and Oura, H. : Biochemical features induced by adenine feeding in rats. Polyuria,

electrolyte disorders, and 2,8-dihydroxyadenine deposits. *J. Nutr. Sci. Vitaminol.* **30**, 245-254, 1984

8) Oura, H., Yokozawa, T., Zheng, P.D. and Koizumi, F. : Adenine-induced chronic renal failure in rats. *Igaku No Ayumi* **130**, 729-730, 1984

9) Yokozawa, T., Zheng, P.D., Oura, H. and Koizumi, F. : Animal model of adenine-induced chronic renal failure in rats. submitted to *Nephron*

10) Sasaki, T. : Urea. In "Rinsyo Kagaku Bunseki II" (Ed. by M. Saito, M. Kitamura and M. Niwa), Tokyo Kagaku Dojin, Tokyo, p 1-33, 1979

11) Murakawa, K. : Creatine and creatinine. In "Rinsyo Kagaku Bunseki II" (Ed. by M. Saito, M. Kitamura and M. Niwa), Tokyo Kagaku Dojin, Tokyo, p 67-87, 1979

12) Samejima, K. and Kitamura, M. : Calcium. In "Rinsyo Kagaku Bunseki V" (Ed. by M. Kitamura, M. Saito and M. Niwa), Tokyo Kagaku Dojin, Tokyo, p 53-90, 1973

13) Wajima, T. : Inorganic phosphate. In "Rinsyo Kagaku Bunseki V" (Ed. by M. Kitamura, M. Saito and M. Niwa), Tokyo Kagaku Dojin, Tokyo, p 116-132, 1973

14) Yokozawa, T., Suzuki, N., Zheng, P.D., Oura, H. and Nishioka, I. : Effect of orally administered rhubarb extract in rats with chronic renal failure. *Chem. Pharm. Bull.* **32**, 4506-4513, 1984

15) Yokozawa, T., Suzuki, N., Okuda, I., Oura, H. and Nishioka, I. : Uremia-preventive effect of rhubarb extract in rats. *J. Med. Pharm. Soc. for WAKAN-YAKU* **2**, 344-350, 1985

16) Oura, H., Zheng, P.D. and Yokozawa, T. : Effect of onpi-tô in rats with chronic renal failure. *J. Med. Pharm. Soc. for WAKAN-YAKU* **1**, 209-217, 1984

17) Oura, H., Chung, H.Y., Zheng, P.D., Yokozawa, T., Wakaki, K. and Koizumi, F. : Effect of onpi-tô administered orally for a long term on rats with chronic renal failure. *J. Med. Pharm. Soc. for WAKAN-YAKU* **2**, 365-371, 1985

18) Oura, H., Chung, H.Y. and Yokozawa, T. : Effect of each component crude drug of the traditional Chinese prescription "onpi-tô" on rats with chronic renal failure. *J. Med. Pharm. Soc. for WAKAN-YAKU* **2**, 351-356, 1985

19) Sasaki, M., Takahara, K. and Natelson, S. : Urinary guanidinoacetate/guanidinosuccinate ratio ; an indicator of kidney dysfunction. *Clin. Chem.* **19**, 315-321, 1973

20) Tofuku, Y., Muramoto, H., Kuroda, M. and Takeda, R. : Impaired metabolism of guanidinoacetic acid in uremia. *Nephron* **41**, 174-178, 1985

21) Yokozawa, T., Chung, H.Y. and Oura, H. : Effect of extract from salviae miltiorrhizae radix on the urinary urea, creatinine, and electrolyte excretion. *J. Med. Pharm. Soc. for WAKAN-YAKU*, in press

22) Salviae miltiorrhizae radix. In "Kanyaku no Rinsyo Ohyo" (Ed. by Chuzan Igakuin), Ishiyaku Shutsupan, Tokyo, p 257, 1980

23) Zhang, J.R., Zheng, X.R., Yang, H.T., Yan, P.Z. and Chen, H. H. : Dan shen (Salvia miltiorrhiza Bunge) therapy in 48 hospital cases of chronic renal insufficiency. *Shanghai J. Traditional Chinese Medicine*, 17-18, 1981