[Chem. Pharm. Bull. 34(9)3818—3822(1986)]

Effect of Extract from Salviae Miltiorrhizae Radix on Uremic Rats

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(Received March 1, 1986)

The effect of Salviae Miltiorrhizae Radix extract on blood constituents was investigated in rats made uremic by feeding an adenine-containing diet. Administration of Salviae Miltiorrhizae Radix extract for 18 d after feeding of the adenine diet for 6 d led to significant reductions of serum urea nitrogen, creatinine, methylguanidine, and guanidinosuccinic acid and an increase of guanidinoacetic acid, suggesting alleviation of the uremic state. In a group fed the adenine diet for 12 d, followed by the extract, there were significant decreases in urea nitrogen and guanidinosuccinic acid. However, the extract no longer caused any reduction of urea nitrogen, creatinine, methylguanidine, or guanidinosuccinic acid, or an increase of guanidinoacetic acid, when the adenine diet was given for 18 d to cause a more severely uremic state.

Keywords——Salviae Miltiorrhizae Radix; uremic rat; urea nitrogen; creatinine; methylguanidine; guanidinosuccinic acid; guanidinoacetic acid

Salviae Miltiorrhizae Radix, a well-known traditional Chinese medicinal herb used to improve the blood circulation and relieve stasis, is widely used by many clinicians in China.[1] It has recently been reported to show vasodilative, hypotensive, anticoagulant, and antibacterial activity[1] and to have a beneficial effect in patients with chronic renal failure.[2] On the other hand, we previously reported that the administration of Salviae Miltiorrhizae Radix extract to adenine-fed rats resulted in significant decreases of urea nitrogen, creatinine, methylguanidine, guanidinosuccinic acid and an increase of guanidinoacetic acid in the serum, which suggested alleviation of the uremic state.[3] In the present paper, the effect of Salviae Miltiorrhizae Radix extract on blood urea nitrogen, creatinine, and guanidino compounds was investigated to examine its availability as a therapeutic agent, in rats made uremic by administration of an adenine-containing diet.

Materials and Methods

Animals and Treatments——Male Wistar-strain rats of 5 weeks of age, initially weighing 110—120 g, were used in the experiment. The animals were fed *ad libitum* on 18% casein diet containing 0.75% adenine. 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 the level of 0.75 g/100 g of the diet. The adenine feeding procedure produced experimental chronic renal failure, as reported previously.[5-8] Administration of the adenine diet for 6 d (experiment 1), 12 d (experiment 2), or 18 d (experiment 3) was followed by administration for 18 or 12 d of Salviae Miltiorrhizae Radix extract, which was allowed *ad libitum* at the concentration of Salviae Miltiorrhizae Radix, 1 mg/ml in water, while control rats were given tap water. The dose of Salviae Miltiorrhizae Radix extract was about 30 mg/rat/d during the experimental period. There were no statistically significant differences between the control and Salviae Miltiorrhizae Radix extract-treated groups with regard to body weight. On the 6th, 12th, or 18th day of administration of the Salviae Miltiorrhizae Radix extract, blood was collected by heart puncture under sodium pentobarbital anesthesia. The blood was allowed to clot at room temperature and then centrifuged. The sera obtained were used for the determination of urea nitrogen. On the last

day of the Salviae Miltiorrhizae Radix extract treatment, rats were stunned by means of a sharp blow on the head and blood samples were collected in a conical centrifuge tube for the determination of creatinine and guanidino compounds.

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 °C for 40 min (roots: water = 1 : 10, w/v), as described previously.[3] The aqueous extract was filtered through 4 layers of gauze and the filtrate was freeze-dried under reduced pressure to provide a brown residue in about 25% yield.

Analyses——Urea nitrogen was determined by using a commercial reagent (BUN KAINOS obtained from Kainos Laboratories, Inc., Tokyo, Japan) based on the urease–indophenol method.[9] Creatinine was determined by using a commercial reagent (Creatinine-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the Folin–Wu method.[10] 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 $1000 \times g$ for 10 min was applied to a Shimadzu LC-5A liquid chromatograph using a stepwise gradient. A fluorescence spectrometer, model RF-540 (excitation 395 nm, emission 500 nm; Shimadzu Co.) was used to monitor the effluent from the column.

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

Urea Nitrogen

The administration of Salviae Miltiorrhizae Radix extract for 18 d after the adenine diet had been given for 6 d (experiment 1) led to a reduction in serum urea nitrogen. As shown in Fig. 1, serum urea nitrogen was significantly decreased in the Salviae Miltiorrhizae Radix extract-treated group on the 18th day of extract treatment. The serum urea nitrogen was also decreased by 12% on the 12th day as compared with the control group. In experiment 2, Salviae Miltiorrhizae Radix extract was administered for 18 d orally, after the adenine diet had been given for 12 d. As shown in Fig. 1, Salviae Miltiorrhizae Radix extract significantly reduced the urea nitrogen level by 20—26% on the 12th and 18th days. On the other hand, when adenine diet was given for 18 d (experiment 3), the reduction of urea nitrogen in the serum was no longer found (Fig. 1).

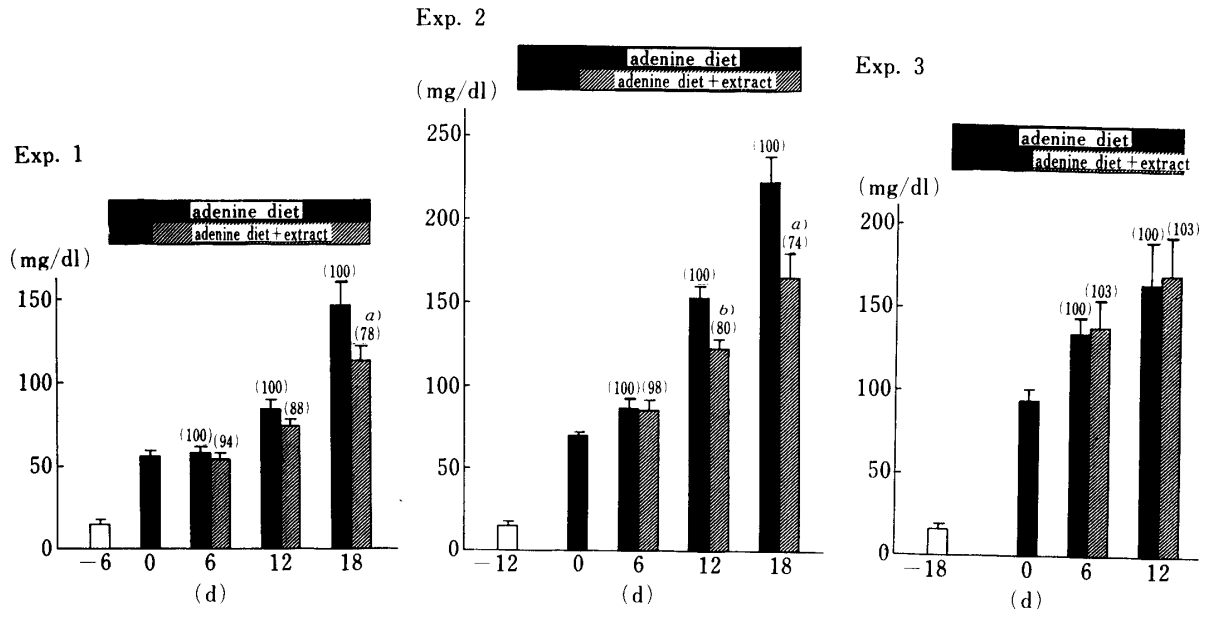

Fig. 1. Effect of Extract from Salviae Miltiorrhizae Radix on Serum Urea Nitrogen

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

Table I. Effect of Extract from Salviae Miltiorrhizae Radix on Serum Creatinine Level

Exp. No.	Material	Creatinine (mg/dl)
1	Control	3.63±0.14 (100)
	Salviae Miltiorrhizae Radix extract	3.01±0.28[a] (83)
2	Control	4.34±0.14 (100)
	Salviae Miltiorrhizae Radix extract	4.05±0.11 (93)
3	Control	3.92±1.22 (100)
	Salviae Miltiorrhizae Radix extract	2.47±0.14 (63)

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 II. Effect of Extract from Salviae Miltiorrhizae Radix on Serum Levels of Guanidino Compounds

Exp. No.	Material	MG (μg/dl)	GSA (μg/dl)	GAA (μg/dl)
1	Control	15.14±1.79 (100)	104.23±18.18 (100)	72.78±9.15 (100)
	Salviae Miltiorrhizae Radix extract	9.00±0.43[b] (59)	77.27±3.77[a] (74)	94.76±4.94[a] (130)
2	Control	31.40±3.78 (100)	117.44±17.65 (100)	84.47±3.24 (100)
	Salviae Miltiorrhizae Radix extract	25.66±2.52 (82)	59.54±4.26[b] (51)	86.25±6.34 (102)
3	Control	22.66±0.99 (100)	154.21±27.05 (100)	60.98±5.32 (100)
	Salviae Miltiorrhizae Radix extract	25.91±3.86 (114)	152.55±23.67 (99)	66.20±2.34 (109)

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

Creatinine

Table I shows the creatinine level in the serum. In experiment 1, the extract from Salviae Miltiorrhizae Radix significantly reduced the creatinine level by 17% as compared with the control. In contrast, administration of the Salviae Miltiorrhizae Radix extract had a lesser effect in experiments 2 and 3. As shown in Table I, some decrease (7—37% compared with the control) in the creatinine level was observed in experiments 2 and 3 but it was not statistically significant.

Guanidino Compounds

As shown in Table II, serum methylguanidine (MG) in the group administered Salviae Miltiorrhizae Radix extract for 18 d after the adenine diet had been given for 6 d, was 41% lower than in the control group. The serum guanidinosuccinic acid (GSA) in the group treated with Salviae Miltiorrhizae Radix extract was also decreased significantly. On the other hand,

the Salviae Miltiorrhizae Radix extract caused a significant increase of 30% in serum guanidinoacetic acid (GAA) concentration (experiment 1). A significant reduction of GSA concentration was observed in experiment 2. However, the administration of Salviae Miltiorrhizae Radix for 12 d after the adenine diet had been given for 18 d showed no appreciable difference in MG, GSA, or GAA as compared with the control.

Discussion

As reported previously,[3] continuous and simultaneous administration of adenine and Salviae Miltiorrhizae Radix extract resulted in reductions of serum urea nitrogen, creatinine, phosphate, and GSA, disappearance of serum MG, and increases of GAA, glycine, serine, glutamic acid, aspartic acid, isoleucine, *etc.* In the present study, the effect of Salviae Miltiorrhizae Radix extract on blood constituents was investigated, after adenine had been given to make rats uremic.

Administration of the extract of Salviae Miltiorrhizae Radix for 18 d after the adenine diet had been given for 6 d led to reductions in urea nitrogen, creatinine, MG, and GSA. In the group given the adenine diet for 12 d followed by the extract, there were significant decreases in blood urea nitrogen and GSA. These effects of Salviae Miltiorrhizae Radix extract after uremia had been induced (experiments 1 and 2) were in agreement with those which were observed in rats given adenine and the extract at the same time.[3] On the other hand, when the adenine diet was given for 18 d, inducing a more severe state of renal failure biochemically and histologically,[5] the reductions of blood urea nitrogen, creatinine, MG, and GSA was no longer found.

Among substances derived from the urea cycle which seem to be uremic toxins, MG and GSA are markedly increased in renal failure, and they have been reported to show various toxic activities such as platelet function disturbance, hemolytic activity, glucose metabolism disturbance, and inhibition of lymphocyte transformation.[11-15] The Salviae Miltiorrhizae Radix extract reduced the accumulation in the body of nitrogen compounds such as MG and GSA, which are increased in uremia, which might be suggestive of a certain benefical effect, alleviating the uremic state.

On the other hand, serum GAA level, which is lowered in uremia, was elevated by administering Salviae Miltiorrhizae Radix extract (experiment 1). Tofuku *et al.*[16] have reported that the glycine amidinotransferase (GAT) activity in the kidney decreases as blood urea nitrogen rises in the course of renal damage, resulting in a lower content of serum GAA in the uremic state. They have also suggested that some toxic substances which may inhibit renal GAT activity are dialyzable. The GAA elevation by Salviae Miltiorrhizae Radix extract may be associated with a decrease of some toxic substances which inhibit renal GAT activity. Further studies on the mechanism of GAA elevation are in progress.

Based on the above findings, it is likely that Salviae Miltiorrhizae Radix extract can delay the progress of uremia. Though further studies remain to be made of the mechanism, Salviae Miltiorrhizae Radix extract seems to have an action which partially ameliorates renal dysfunction, in view of the interesting information that this extract accelerates the elimination of urea and creatinine into urine.[17] From the above observations, it is proposed that Salviae Miltiorrhizae Radix has a beneficial effect on uremia, possibly as a result of hyperfiltration in remnant nephrons still functioning.

Zhang *et al.*[2] have recently reported that an intravenous drip of Salviae Miltiorrhizae Radix for the therapy of chronic renal failure patients has a beneficial effect, which is manifested as a significant reduction in blood urea nitrogen and serum creatinine, with an increase of creatinine clearance clinically. These clinical findings are supported in part by the uremia alleviating effect of Salviae Miltiorrhizae Radix which we have observed in the present

experiment.

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