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Toxic Effects of *Polygalae Radix* on Rat Kidney

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ABSTRACT : *The renal toxicity of the extract of Polygalae Radix was investigated in rats. Rats were treated with 3.5 mg/Kg of the extract, i.p., for 7 days. Changes in consumatory behavior, 24 hour-urine and the activities of urinary enzymes were determined during the administration of the extract. Significant decrease in body weight and food consumption and increase in 24 hour-urine volume were observed during the administration. However, the quantity of total creatinine in urine was decreased significantly. Those indicate that subacute treatment with the extract might induce diuresis and the diuresis might be due to the decrease in water reabsorption. In the activities of urinary enzymes, the activities of alanine aminopeptidase (AAP) and gamma-glutamyl transpeptidase (GGT) were increased 4.3 and 3.5 times and then returned to the control. The activity of N-acetyl-β-D-glucosaminidase (NAG) was increased 7.2 times and then decreased slowly. But, it was significantly higher than that of the control even after the last administration. The activity of lactate dehydrogenase (LDH) was increased continuously during the treatment. It showed 32 times higher than the control. These results suggested that the extract of Polygalae Radix had toxic effect on kidney. Furthermore, the result suggested that the subacute administration of the extract induced resistance against the toxicity of Polygalae Radix.*

Key Words : *Polygalae Radix, Creatinine, Alanine-aminopeptidase, Gamma-glutamyl transpeptidase, N-acetyl-β-D-glucosaminidase, Lactate dehydrogenase*

I. INTRODUCTION

Polygalae radix (Polygala tenuifolia, Polygalaceae), one of the herbal medicines recorded in ancient pharmacopoeia, has been administered as a sedative or an expectorant for a long time. It contains polygalitol, xanthones, onjisaponins, alkaloids and etc (Bashir *et al.*, 1991; Han *et al.*, 1985). The pharmacological effects such as sedation, diuresis, hemolysis, contraction of uterus and CNS supression have been reported (Park, 1983). Sordat-Diserens *et al.* (1991) reported antifungal effect of xanthone components. It has also been reported that crude saponin of *Polygalae Radix* increases urine volume and quantity of electrolytes released in urine (Park, 1983). Furthermore, Park suggested that the diuretic effect might be caused by vasodilation of glomerulus. However, diuresis can be caused by several other factors, such as abnormality of glomerulus, hormones or neural activities (Ganong, 1989; Lim *et al.*, 1992).

It has been reported that total level of some urinary enzymes increases in the damaged kidney. Activities of the enzymes in the urine can be used as an indicator of renal damage without injury to the examinee (Hofmeister *et al.*, 1986; Ohata *et al.*, 1987; Wachsmuth, 1982).

Herbal medicines generally remedy a disease through long term administration. However, researchs about subacute effects of herbal medicines are insufficient compared to the ones about acute effects.

Therefore, we undertook a systemic investigation of changes in consumatory behaviors, 24 hour-urine volume and activities of various urinary enzymes during the administration of the extract of *Polygalae Radix*.

II. METHODS

1. Animal and Materials

Male Spraque-Dawley rats weighing 250-350 g, housed under 12 hour light/dark cycle, 23±1°C ,

60±5% humidity, were used. All animals had free access to food and water. MPS-1 (micropartition system-1) and membrane were purchased from Amicon (Denver, CO.). The diagnostic kit for GGT (γGT 14) was purchased from Gilford (Cleveland, OH). The other chemicals were purchased from Sigma (St. Louise, MO).

2. Extraction of *Polygalae Radix*

Crude drug cut as small as possible, was soaked in methanol for 24 hours and then refluxed in a water bath for 2.5 hours. When this fluid was warm, it was filtered. The same extraction procedures were repeated twice and each filtrate was mixed with the first extraction filtrate. Then, the whole filtrate was vaccum-evaporated and freeze-dried. After the dried material was dissolved in distilled water, the water fraction was separated and vaccum-dried.

3. Treatment of Animal

The dried fraction was dissolved in saline and the various doses of the extract were administered, i.p., to the animals. The lithality for 24 hour was observed and lethal dose (LD_{50}) was calculated. For the study of metabolic changes, animals were adapted in metabolic cages for 3-4 days. During the administration of the extract 3.5 mg/Kg, i.p., for 7 days, changes in body weight, food and water consumption, and 24hour-urine volume were determined at each rat. Also, the kidneys were removed and weighed after the acute (10 mg/Kg, once) and subacute (4 mg/Kg, daily for 7 days) administrations.

4. Pretreatment of Urine for Enzyme Determinations

It has been reported that enzyme inhibitors are contained in urine. To remove the inhibitors, micropartition systems were employed (Leathwood *et al.*, 1969; Ohata *et al.*, 1987); 24 hour-collected urine was centrifuged at 800×g, 4°C for 5 min. The diluted supernatant was added to MPS-1 and centrifuged at 1500×g, 5°C for 50 min. The filtrate in the lower tube of MPS-1 was used for creatinine quantification. To dissolve the enzymes attached to the membrane, phosphate buffered saline (PBS, 50 mM KH_2PO_4-K_2HPO_4 in saline, pH 7.4) was applied to the upper tube of MPS-1 and vortexed. After the same procedure was repeated, the wash was mixed with the first fluid. Then it was used as the source for enzyme determinations. Enzyme activities were represented as creatinine ratios.

5. Creatinine Measurement

It was determined by Jaffe reaction (Rock *et al.*, 1987); The diluted filtrate 1.5 ml and 0.36 M picric acid 0.5 ml were mixed. After 30 sec, 1.4 M NaOH was added to stop the reaction and 15 min stabilization followed. The detection wavelength for quantification was 500 nm.

6. N-acetyl-β-D-glucosaminidase Activity (NAG)

It was determined by Maruhn method (1976); 0.25 ml of substrate (5 mM p-nitrophenol-N-Acetyl-β-glucosaminide in 50 mM Citric acid-K_2HPO_4-KOH buffer, pH 4.2) was added to 0.05 ml of enzyme sample. After 40 min incubation at 37°C, the reaction was stopped by adding 0.1 M borate buffer (H_3PO_4-KOH, pH 10.5) and the absorbance at 406 nm was determined.

7. Alanine Aminopeptidase Activity (AAP)

It was determined by Jung and Scholz method (1980); 0.08 ml of enzyme sample and 0.8 ml of substrate (2 mM L-alanine-4-nitroanilide) were incubated at 37°C for 20 min. The reaction was stopped by adding 20% sodium dodecyl sulfate (SDS) and the absorbance at 406 nm was determined.

8. Gamma-glutamyl Transpeptidase Activity (GGT)

It was determined by Szasz method (1974); The diagnostic kit for GGT was applied to the 0.02 ml of enzyme sample. After 10 min incubaton at 25°C, the reaction was stopped by adding 20% SDS and the absorbance at 406 nm was determined.

9. Lactate Dehydogenase Activity (LDH)

It was determined by Bergmeyer and Bernt method (1974); Enzyme sample was incubated with NADH (1 mg/ml) and phosphate buffer (0.1 M K_2HPO_4-KH_2PO_4 containing 0.2% Triton X-100, pH 7.4) at 30°C for 5 min. The decreased optical density at 340 nm during the incubation was used to calculate the activity.

10. Statistics

Statistical significance was determined by Student's t-test. Lethal dose after i.p. administration of the extract was calculated by Quantal-Dose Response method.

III. RESULT

1. Changes in Body Weight and Metabolic Rate

LD_{50} after i.p. injection of the *Polygalae Radix* extract was 32.3 mg/Kg (95% confidence range 13.1-79.7). Changes in body weight during the administration of the extract, 3.5 mg/Kg, i.p., for 7days were shown in Fig. 1. All the animals treated at this dose survived through the period but their body weight decreased significantly. Changes in consumatory behavior during the subacute administration were shown in Fig. 2. Water consumption increased twice after the 3rd-day administration. Food consumption continuously decreased and became half of the control after the 3rd-day administration.

Twenty four hour-urine volume during the administration was shown in Fig. 3. It was increased twice and then gradually decreased. Even on the 7th-day, the value was still significantly higher than the control.

After the acute and subacute administrations, the kidneys were removed and weighed. The kidney expansion was observed and their weight per body weight significantly incereased (Table 1).

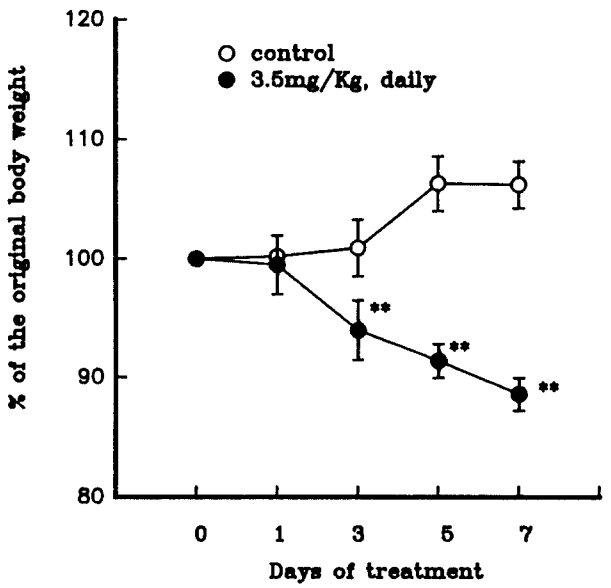

Fig. 1. Changes in the body weight during the administration of the *Polygalae Radix* extract (3.5 mg/Kg, daily). The values represents mean±S.E. for 4 or 5 animals. The 100% value represents the body weight before the administration. ** means significantly different from the control value at $p < 0.01$.

2. Creatinine Concentration and Urinary Enzyme Activities

Changes in the total creatinine of the 24 hour-urine during the subacute administration of the *Polygalae Radix* extract were shown at Fig. 4. The released amount was continuously decreased. After the 3rd-day administration, siginificantly small

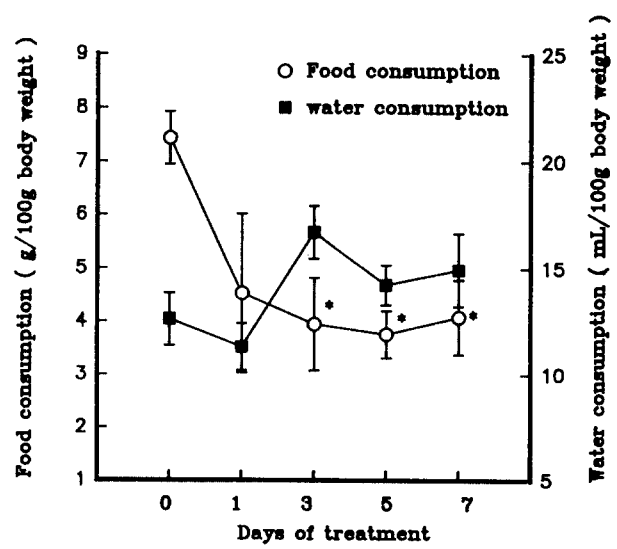

Fig. 2. Changes in consumatory behaviors durine the administration of the *Polygalae Radix* extract (3.5 mg/Kg, daily). The value represents mean±S.E. for 4 animals. * means significantly different from the control at $p < 0.05$.

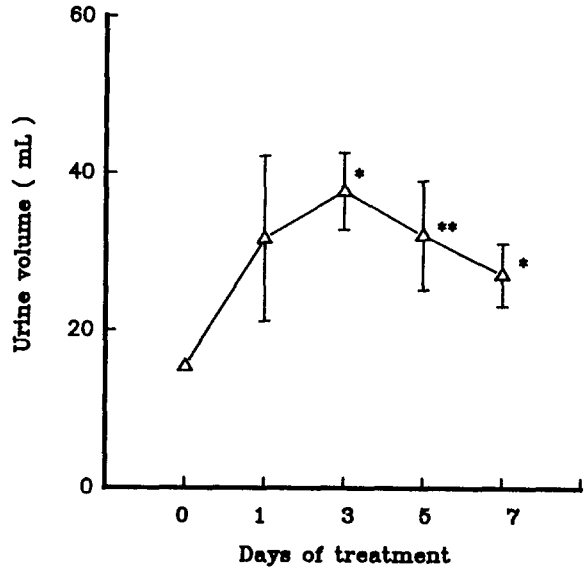

Fig. 3. Changes in the excreted urine volume during the administration of the *Polygalae Radix* extract (3.5 mg/Kg, daily). The values represents mean±S.E. for 3 or 4 determinations. * significantly different from the control at $p < 0.05$. ** significantly different from the control at $p < 0.01$.

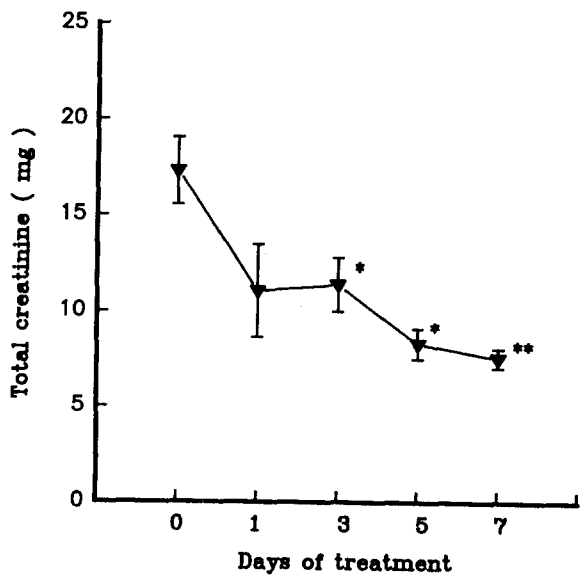

Fig. 4 Changes in the total creatinine released in the 24 hour-urine during the administration of the *Polygalae Radix* extract (3.5 mg/Kg, daily). Legends are the same as Fig. 3.

Table 1. Kidney weight

control	acute	subacute
0.77±0.02	0.88±0.04*	1.07±0.06**

The values represent mean±S.E. (kidney weight g/100 g body weight) for 4 determinations. The acute and subacute groups were treated with the extract of *Polygalae Radix* 10 mg/Kg, once and 4 mg/Kg, daily for 7 days, respectively. * significantly different from the control at $p < 0.05$. ** significantly different from the control at $p < 0.01$.

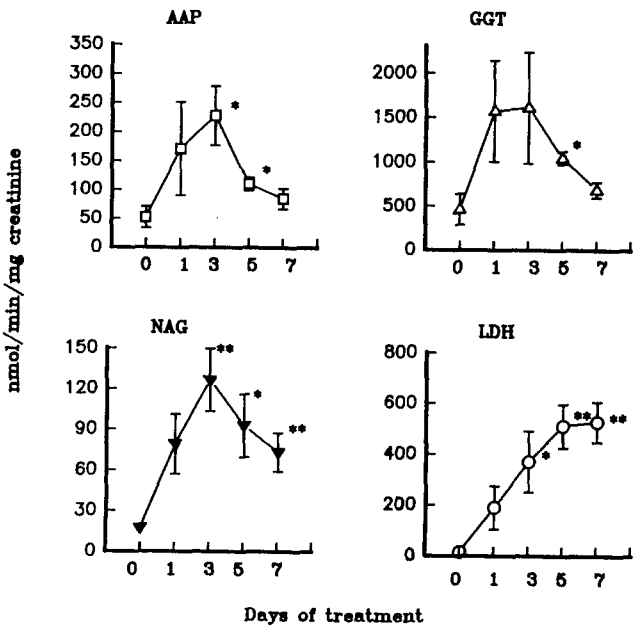

Fig. 5. Changes in the activities of several urinary enzymes during the administration of the *Polygalae Radix* extract (3.5 mg/Kg, daily). Legends are the same as Fig. 3.

amount of creatinine was released.

The activities of AAP, NAG, GGT, LDH were shown at Fig. 5. The AAP and GGT activities showed the highest value after the 3rd-day administration and decreased to the control value on the last day. The NAG activity also showed the highest value after the 3rd-day administration but didn't return to the control value. The LDH activity increased in time dependent manner. After the 5th and 7th-day administration, it showed 31-32 times higher value than the control.

IV. DISCUSSION

The present results demonstrate that the treatment of *Polygalae Radix* in rats induces renal impairment. The excretion of urine was increased and the various urinary enzyme activities were increased. However, the release of creatinine was decreased.

It has been reported that LD_{50} of *Polygalae Radix* following s.c. and p.o. administration on mice were 71.1 mg/Kg and 694.5 mg/Kg respsctively (Park,

1983). Relatively low LD_{50} value in our result might be due to the difference in administration route.

The brain region which controls the consumatory behavior is hypothalamus. However, it is known that food consumption and water consumption are controlled by different nervous systems. Russell *et al.* (1969) reported that water consumption was more related to the cholinergic nervous system than food consumption. At the present result, urine volume was increased during the administration. Thus, the increased urine excretion might induce the increase of water consumption. Body weight reduction, changes in the consumatory behavior, renal expansion might indicate that certain components of *Polygalae Radix* induce metabolic imbalance and renal impairment.

It has been reported that urine volume of rabbit was increased by the acute administration of *Polygalae Radix* and release of the electrolytes such as Na^+, K^+ and Cl^- was also increased. Creatinine clearance was increased (Park, 1983). However, the present results indicate that creatinine release in 24 hour-urine continuously decreased and showed significantly low values after the 3rd-day administration. It has been reported that plasma creatinine concentration can be influenced by the size of muscle or diet (Hoffmann *et al.*, 1981; Pfeifer *et al.*, 1975; Shin *et al.*, 1989). Thus, the reduced amount of urinary creatinine was partly due to their reduced body weights and the reduced food consumption. Also, it has been reported that glomerular filtration of creatinine can also be altered by hormonal or neuronal signals (Lee, 1986). Although more studies of *Polygalae Radix* on hormonal or neuronal effects are needed, the administration of *Polygalae Radix* might induce short-term increase and long-term decrease of glomerular filtration. Thus, the diuresis might be due to the decrease in water reabsorption.

Activities of several urinary enzymes have been used as indicators of the kidney injuries. It has been reported that most renal toxic substances induce necrosis in proximal tubular cells, the lysosomal and cytoplasmic enzymes such as NAG, AAP, GGT, LDH would leak into the urine (Ohata *et al.*, 1987; Shin *et al.*, 1990). Furthermore, Harauchi and Yoshizaki (1990) reported that the urinary enzyme activities represented as creatinine ratio were more uniform than the total activities. However, our results showed no considerable difference between them. Changes in urinary enzymes during the subacute administration of *Polygalae Radix* were similar to those after the acute administration of cephaloridine reported by Harauchi and Yoshizaki (1990). The activities of AAP and GGT, rich in brush border, were increased until the 3rd-day administration and then returned to the control value soon. A lysosomal enzyme, NAG, showed similar trends but more slow recovery than AAP and GGT did. These imply that continuous administration of *Polygalae Radix* doesn't induce additive toxicity. However, a cytoplasmic enzyme, LDH, showed continuously high urinary activity during the administration. This implies that the subacute administration induces the damage in renal cell membrane. Although the chronic toxicity study is needed to further illustrate, the results suggest that the administration of *Polygalae Radix* might differently affect cellular damage; the acute exposure might disturb the transient cellular homeostasis and the subacute exposure might induce the damage in the renal cell membrane.

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