

## Heat-processed ginseng saponin ameliorates the adenine-induced renal failure in rats

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To evaluate the effect of the saponin of heat-processed ginseng (Sun ginseng, SG), we investigated the protective effect of SG total saponin fraction against adenine-induced chronic renal failure in rats. SG saponin significantly decreased the levels of urea nitrogen and creatinine in the serum, but increased the urinary excretion of urea nitrogen and creatinine, indicating an improvement of renal function. SG saponin also inhibited adenine-induced kidney hypertrophy and edema. SG saponin reduced serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and lactate dehydrogenase activities increased by adenine. Based on these findings, the ameliorating effect of SG on chronic renal failure may result from its saponin.

**Keywords:** *Panax ginseng*, Sun ginseng, Ginseng, Saponin, Renal insufficiency

### INTRODUCTION

Chronic renal disease (CRD) is a global public health problem and now the leading causes of death worldwide [1]. The World Health Organization estimates that there were approximately 58 million deaths worldwide, with 35 million attributed to chronic disease in 2005 [2,3]. CRD causes many complications such as anemia, pericarditis or cardiovascular disease, etc. End stage of renal disease is managed by dialysis, renal transplant or supportive end of life care. Therefore, alternative approaches would reduce the cost of dialysis, drug therapy and renal transplantations [4]. One of such approach has recently been suggested by herb medicines such as rhubarb, ginseng, etc. [5-7].

Ginseng (the root of *Panax ginseng* Meyer, Araliaceae) has been frequently used in Asian countries for cancer, inflammation, stress, etc. [8,9]. To enhance the pharmacological activities of ginseng, steaming and heating process have been adopted for ginseng. Sun

ginseng (SG) is prepared by steaming fresh ginseng at a higher temperature than red ginseng [10,11]. Its main components are ginsenoside Rg3, Rg5, and Rk1. These ginsenosides show anticancer, anti-inflammatory, anti-dermatitic, platelet anti-aggregating, radical scavenging, and neuroprotective activities [12,13]. In addition, we recently found that SG ameliorates the adenine-induced chronic renal failure in rats [14]. Feeding of adenine diet produced experimental animal model for chronic renal failure, and it is an established animal model of human CRD [15,16]. However, the effect of its saponin on renal failure was not studied.

Therefore, we obtained total saponin extract of SG by fractionating with *n*-BuOH to efficiently extract the saponins [17], and investigated its protective effects in the adenine-induced renal failure rats through biochemical measurements in plasma and urine.

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## MATERIALS AND METHODS

### Materials

Adenine and casein were obtained from Sigma Chemical (St. Louis, Mo, USA). Alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, blood urea nitrogen and creatinine assay kits were purchased from Asan Pharmaceutical (Seoul, Korea). SG extracts (water extract of Sun ginseng [SGW] and *n*-BuOH fraction of Sun ginseng [SGB]) were provided from Ginseng Science (Seoul, Korea). In short, SGW is obtained through the process of extracting SG with water, evaporating and lyophilizing. And SGB is obtained through the process of fractionating SGW by *n*-butanol, evaporating and lyophilizing.

### Animals

Male Sprague Dawley rats (6 wk, 190-210 g) were obtained from Samtako Biokorea (Seoul, Korea). All animals were fed on standard laboratory chow (Samyang, Seoul, Korea), housed in wire cages at 24±2°C and 50±10% humidity and allowed to water *ad libitum*. All experiments were performed in accordance with the National Institutes of Health and Kyung Hee University guides for Laboratory Animals Care and Use and approved by the Institutional Animal Care and Use Committee in the Kyung Hee Medical Center, Kyung Hee University.

### Experimental design

Adenine-induced chronic renal failure rat model, which showed diffuse tubular injury with neutrophil polymorph infiltration, tubular necrosis, tubular atrophy and diffuse intestinal fibrosis [18], was performed according to the method of Yokozawa et al. [6,19]. Briefly, the animals were fed on 18% casein diet containing 0.75% adenine. The composition of diet is as follow (in 100 g): casein 18 g,  $\alpha$ -cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture 4 g, vitamin mixture 1 g, cellulose powder 2 g, choline chloride 0.1 g, and adenine 0.75 g. This procedure of adenine feeding produced experimental chronic renal failure.

Rats were randomly divided into six groups of 7 rats in each group. Normal group had free access to normal diet. The other five groups were fed with 18 % casein diet containing 0.75% adenine. And SGW-50, 100 and SGB-25, 50 groups were fed with adenine diet and SGW (50, 100 mg/kg, per os) or SGB (25, 50 mg/kg, per os) once daily for 20 d, respectively.

### Measurement of blood pressure and heart pulse rate

On the 20 d after the administration of adenine, blood pressure and heart pulse rate of rats were measured by a non-invasive method using an automatic blood pressure monitor. After being warm at constant temperature (37°C) for 15 min, a tail artery blood pressure and heart rate were measured and compared.

### Blood and urine collection

Blood samples of rats were obtained on the 10th and 20th days after the administration of adenine. Blood samples were collected by the cardiac puncture and centrifuged at 3,000 xg for 20 min at 4°C. And they were used to measure urea nitrogen, creatinine, calcium and phosphate levels and glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) activities. Urine samples of each rat were collected in metabolic cages for 24 h on the 9th and 19th days after the administration of adenine. And they were used to measure urea nitrogen, creatinine, calcium and phosphate levels.

### Measurement of urea nitrogen, creatinine, calcium and phosphate levels, and GOT, GPT, and LDH activities

The levels of urea nitrogen and creatinine in the blood serum and urine samples were determined by Urease-Indophenol method [20] and Jaffe method [21], respectively. The levels of calcium and phosphate in the blood serum and urine samples were determined by *o*-cresolphthalein complexone method [22] and Goldenberg method [23] using Hitachi automatic analyzer. The activities of serum transaminase (GOT and GPT) and LDH were determined by Reitman and Frankel method [24] and Wroblewski method [25], respectively.

### Measurement of the body, kidney, and liver weight

The body weight was recorded four times at intervals of five d after the administration of adenine. The kidney and liver weight were measured after collecting blood serum and urine samples on the 20th day.

### Statistical analysis

All values are expressed as mean±SEM. Data analysis was determined significant at the level of  $p < 0.05$  using student's *t*-test.

## RESULTS AND DISCUSSION

To evaluate the protective effect of SG saponin on

**Table 1.** Effect of SGW and SGB on BUN, serum creatinine, calcium, and phosphate levels in adenine-induced renal failure rats

Groups	Dose (mg/kg, per os)	BUN (mg/dL)		Creatinine (mg/dL)		Calcium (mg/dL)		Phosphate (mg/dL)	
		10 d	20 d	10 d	20 d	10 d	20 d	10 d	20 d
Normal	-	16.5±0.99	14.6±1.09	0.49±0.02	0.56±0.02	10.5±0.04	10.4±0.05	8.9±0.03	8.6±0.01
Control	-	91.5±6.19 <sup>###</sup>	150.2±6.73 <sup>###</sup>	1.14±0.06 <sup>###</sup>	2.14±0.13 <sup>###</sup>	9.6±0.08 <sup>###</sup>	9.6±0.11 <sup>###</sup>	10.2±0.08 <sup>###</sup>	12.0±0.15 <sup>###</sup>
SGW	50	86.8±8.32 (6.2)	131.6±8.56 (13.8)	1.08±0.10 (9.2)	1.90±0.13 (15.1)	9.8±0.12* (22.2)	9.7±0.12 (11.1)	9.5±0.12** (53.8)	10.7±0.11** (38.2)
SGW	100	78.0±2.71* (18.0)	128.9±8.59* (15.7)	0.98±0.07* (25.0)	1.81±0.10* (20.6)	10.1±0.05** (55.5)	9.9±0.13* (22.3)	9.2±0.07*** (76.9)	10.1±0.09** (55.9)
SGB	25	75.2±3.48* (21.7)	126.8±4.65* (17.3)	1.03±0.07 (17.3)	1.88±0.09 (16.7)	9.9±0.03* (33.3)	9.6±0.11 (10.2)	9.4±0.07** (60.5)	10.5±0.07** (44.1)
SGB	50	69.1±5.83** (29.9)	118.2±9.20** (23.6)	0.93±0.07** (32.7)	1.76±0.12* (23.8)	10.3±0.09*** (77.8)	10.1±0.10** (44.5)	9.1±0.09*** (88.5)	9.5±0.11*** (73.5)

Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. The values in parenthesis are % of protection.

#Significantly different from the normal value (<sup>###</sup> $p<0.001$ ), \*significantly different from the control value ( $p<0.05$ , \*\* $p<0.01$ , and \*\*\* $p<0.001$ ). SGW, water extract of Sun ginseng; SGB, *n*-BuOH fraction of Sun ginseng; BUN, blood urea nitrogen.

**Table 2.** Effect of SGW and SGB on serum transaminase activities (GOT and GPT) and LDH activities in adenine-induced renal failure rats

Groups	Dose (mg/kg, per os)	GOT (U/L)		GPT (U/L)		LDH (U/L)	
		10 d	20 d	10 d	20 d	10 d	20 d
Normal	-	120.6±11.32	102.6±13.21	36.1±2.65	32.4±1.57	1,482.0±134.8	1,113.8±66.7
Control	-	192.5±2.55 <sup>###</sup>	148.9±13.51 <sup>###</sup>	43.2±1.15 <sup>###</sup>	38.9±6.43 <sup>###</sup>	2,730.0±234.4 <sup>###</sup>	1,611.4±131.3 <sup>###</sup>
SGW	50	137.1±19.1*** (77.1)	124±18.54** (53.8)	40.9±1.37* (30.1)	36.4±2.59* (38.5)	1,894.4±113.4*** (67.0)	1,370.4±77.5** (48.4)
SGW	100	126.5±17.5*** (91.8)	116.8±15.67*** (69.8)	38.5±5.69** (64.3)	35.4±7.43** (53.8)	1,601.1±116.3*** (90.5)	1,263.5±60.2*** (69.9)
SGB	25	130.3±23.1*** (86.5)	123±13.96** (55.9)	40.6±7.17* (34.3)	36.9±3.46** (41.5)	1,784.4±162.9*** (75.8)	1,245.9±82.0*** (73.4)
SGB	50	123.9±21.6*** (95.4)	105.3±17.87*** (94.2)	37.6±1.09*** (77.1)	35.1±2.67** (58.5)	1,577.4±37.2*** (94.1)	1,169.1±111.0*** (88.9)

Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. The values in parenthesis are % of protection.

#Significantly different from the normal value (<sup>##</sup> $p<0.01$  and <sup>###</sup> $p<0.001$ ), \*significantly different from the control value ( $p<0.05$ , \*\* $p<0.01$ , and \*\*\* $p<0.001$ )

SGW, water extract of Sun ginseng; SGB, *n*-BuOH fraction of Sun ginseng; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactate dehydrogenase.

adenine-induced chronic renal failure in rats, we investigated the effect of SGW and SGB on blood urea nitrogen and serum creatinine, calcium and phosphate levels in adenine-induced renal failure rats (Table 1). Blood urea nitrogen level of control group was increased to 91.5±6.19 mg/dL and 150.2±6.73 mg/dL on the 10th and 20th days after the administration of adenine, respectively. It was significantly increased as compared with that of normal group ( $p<0.001$ ). SGW-100, SGB-25,

and SGB-50 significantly inhibited blood urea nitrogen levels ( $p<0.05$ ,  $p<0.05$ , and  $p<0.01$ ). Treatment with adenine also increased serum creatinine level to 1.14±0.06 mg/dL and 2.14±0.13 mg/dL on the 10th and 20th days after the administration of adenine, respectively. And it was significant as compared with that of normal group (0.49±0.02 mg/dL and 0.56±0.02 mg/dL). SGW and SGB reduced creatinine levels in a dose dependent manner. Especially, SGB-50 significantly reduced creatinine

level to 0.93±0.07 mg/dL and 1.76±0.12 mg/dL on the 10th and 20th days after the administration of adenine, respectively ( $p<0.01$  and  $p<0.05$ ). Also treatment with adenine significantly reduced serum calcium level and increased serum phosphate level ( $p<0.001$ ). SGW and SGB significantly increased calcium levels and inhibited phosphate levels. Like this, we found that SGW and SGB inhibit hematological changes caused by adenine, and SGB containing more saponin had a better effect for inhibiting these changes.

Also, we investigated serum transaminase (GOT and GPT) activities and serum LDH activity of SGW and SGB on adenine-induced renal failure in rats (Table 2). GOT activity of control group was increased to 192.5±2.55 U/L and 148.9±13.51 U/L on the 10th and 20th days after the administration of adenine, respectively. It was significantly increased as compared with that of normal group ( $p<0.001$ ). SGW and SGB decreased GOT activities in a dose dependent manner. Especially, SGB-50 reduced GOT activity to 123.9±21.6 U/L and 105.3±17.87 U/L on the 10th and 20th days ( $p<0.001$ ). Treatment with adenine also significantly increased GPT activity to 43.2±1.15 U/L and 38.9±6.43 U/L on the 10th and 20th days as compared with that of normal group ( $p<0.001$  and  $p<0.01$ ). SGW and SGB decreased GPT activities in a dose dependent manner. SGB-50 reduced GPT activity to 37.6±1.09 U/L and 35.1±2.67 U/L on the 10th and 20th days ( $p<0.001$  and  $p<0.01$ ), respectively. And, treatment with adenine significantly increased LDH activity as compared with that of normal group ( $p<0.001$ ).

SGW and SGB decreased LDH activities. Especially, SGB-50 reduced LDH activity to 1,577.4±37.2 U/L and 1,169.1±111.0 U/L on the 10th and 20th days ( $p<0.001$ ).

In addition, the effects of SGW and SGB on the urine urea nitrogen, creatinine, calcium, and phosphate levels were measured (Table 3). Urine urea nitrogen level of control group was reduced to 710.2±40.9 mg/dL and 720.4±51.1 mg/dL on the 10th and 20th days after the administration of adenine, respectively. And it was significantly decreased in comparison with that of normal group (955.6±61.8 mg/dL and 1072.1±73.2 mg/dL on the 10th and 20th days) ( $p<0.001$ ). SGW and SGB increased urine urea nitrogen level in a dose dependent manner, and the effect of SGB-50 was better than that of the other groups. Treatment with adenine also reduced urine creatinine level, compared with that of normal group ( $p<0.001$ ). SGW and SGB increased creatinine levels in a dose dependent manner. Treatment with adenine significantly increased urine calcium level, meanwhile decreased urine phosphate level compared with that of normal group ( $p<0.001$ ). SGW and SGB inhibited urine calcium levels in a dose dependent manner, but there was no significant effect on the 20th day. On the other hand, SGW and SGB increased urine phosphate levels. Especially, SGB-50 showed a greater increase in this level than the other groups on the 10th and 20th day ( $p<0.01$  and  $p<0.05$ ).

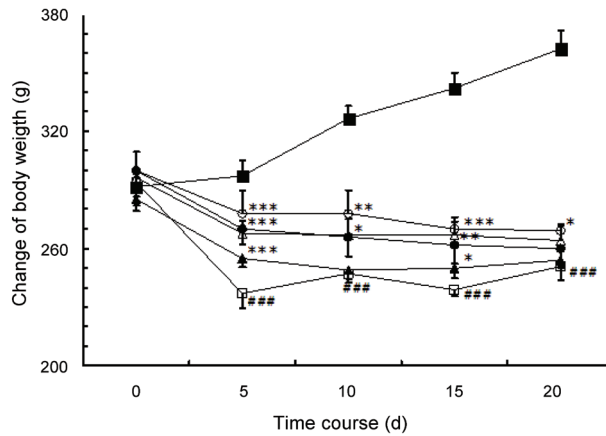
Next, we measured the body weight, kidney and liver weights in adenine-induced renal failure rats. The body weight of normal group was increased by degrees during the experiment period (Fig. 1). However, the body weight

**Table 3.** Effect of SGW and SGB on urine urea nitrogen, creatinine, calcium, and phosphate levels in adenine-induced renal failure rats

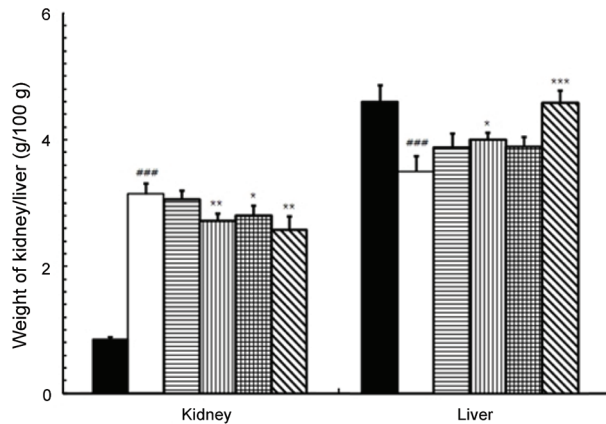
Groups	Dose (mg/kg, per os)	Urea nitrogen (mg/dL)		Creatinine (mg/dL)		Calcium (mg/dL)		Phosphate (mg/dL)	
		10 d	20 d	10 d	20 d	10 d	20 d	10 d	20 d
Normal	-	955.6±61.8	1072.1±73.2	43.5±1.22	50.3±3.12	0.19±0.04	0.18±0.02	31.9±3.53	26.7±2.29
Control	-	710.2±40.9 <sup>###</sup>	720.4±51.1 <sup>###</sup>	16.5±1.06 <sup>###</sup>	12.8±1.32 <sup>###</sup>	0.48±0.06 <sup>###</sup>	0.92±0.17 <sup>###</sup>	20.1±1.51 <sup>###</sup>	14.1±1.83 <sup>###</sup>
SGW	50	745.1±52.7 (14.3)	770.6±41.2 (14.2)	20.5±0.75 (14.8)	15.3±0.59 (6.7)	0.33±0.09* (51.3)	0.80±0.18 (17.1)	22.6±2.08 (21.6)	16.3±0.78 (17.3)
SGW	100	831.7±41.8** (49.4)	826.5±52.4** (30.1)	22.9±0.99* (23.7)	21.2±0.87* (22.4)	0.28±0.07** (67.7)	0.73±0.22 (26.1)	24.8±1.49* (39.9)	17.8±1.68 (29.7)
SGB	25	757.4±31.6* (19.2)	800.4±41.7* (22.7)	20.8±1.15* (15.9)	17.1±1.91 (11.5)	0.27±0.07** (71.8)	0.79±0.20 (17.3)	23.6±2.52 (29.6)	15.5±2.09 (11.5)
SGB	50	850.6±32.1*** (57.1)	890.7±32.5** (48.3)	24.3±2.07** (28.9)	24.1±1.12* (30.1)	0.25±0.03*** (78.5)	0.71±0.12 (28.2)	25.3±2.19** (44.2)	18.5±2.23* (35.4)

Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. The values in parenthesis are % of protection.

<sup>###</sup>Significantly different from the normal value ( $^{###}p<0.001$ ), \*significantly different from the control value ( $^*p<0.05$ ,  $^{**}p<0.01$ , and  $^{***}p<0.001$ ). SGW, water extract of Sun ginseng; SGB, *n*-BuOH fraction of Sun ginseng.

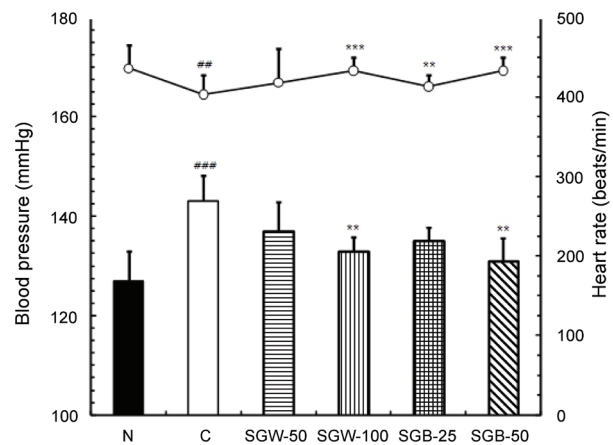


**Fig. 1.** Effect of water extract of Sun ginseng (SGW) and *n*-BuOH fraction of Sun ginseng (SGB) on the body weight in adenine-induced renal failure rats. Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. #Significantly different from the normal value (###*p*<0.001), \*significantly different from the control value (\**p*<0.05, \*\**p*<0.01, and \*\*\**p*<0.001). ■ Normal diet group, □ 0.75% adenine diet group, ▲ 0.75% adenine diet plus 50 mg/kg of the SGW group, △ 0.75% adenine diet plus 100 mg/kg of the SGW group, ● 0.75% adenine diet plus 25 mg/kg of the SGB group, ○ 0.75% adenine diet plus 50 mg/kg of the SGB group.



**Fig. 2.** Effect of water extract of Sun ginseng (SGW) and *n*-BuOH fraction of Sun ginseng (SGB) on the kidney and liver weight in adenine-induced renal failure rats. Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. #Significantly different from the normal value (###*p*<0.001), \*significantly different from the control value (\**p*<0.05, \*\**p*<0.01, and \*\*\**p*<0.001). ■ normal diet group, □ 0.75% adenine diet group, ▲ 0.75% adenine diet plus 50 mg/kg of the SGW group, △ 0.75% adenine diet plus 100 mg/kg of the SGW group, ● 0.75% adenine diet plus 25 mg/kg of the SGB group, ○ 0.75% adenine diet plus 50 mg/kg of the SGB group.

of control group fed on adenine diet was significantly reduced compared with that of normal group (*p*<0.001). SGW and SGB suppressed the adenine-induced body weight loss. Treatment of adenine significantly increased the kidney weight (*p*<0.001), meanwhile SGW and SGB inhibited the weight in dose dependent manner (Fig. 2).



**Fig. 3.** Effect of water extract of Sun ginseng (SGW) and *n*-BuOH fraction of Sun ginseng (SGB) on blood pressure and heart rate in adenine-induced renal failure rats. Sun ginseng (SGW and SGB) were fed to the rats with 0.75% adenine diet once a day for 20 d. Values are expressed as mean±standard error of 7 rats. #Significantly different from the normal value (##*p*<0.01 and ###*p*<0.001), \*significantly different from the control value (\*\**p*<0.01 and \*\*\**p*<0.001). ■ Normal diet group (N), □ 0.75 % adenine diet group (C), ≡ 0.75% adenine diet plus 50 mg/kg of the SGW group (SGW-50), ≡ 0.75% adenine diet plus 100 mg/kg of the SGW group (SGW-100), ≡ 0.75% adenine diet plus 25 mg/kg of the SGB group (SGB-25), ≡ 0.75% adenine diet plus 50 mg/kg of the SGB group (SGB-50).

Also, treatment with adenine significantly reduced liver weight, compared with that of normal group (*p*<0.001) (Fig. 2). SGW and SGB inhibited the liver weight loss. To be short, SGW and SGB more improved the adenine-induced body and liver weight loss, and suppressed the kidney weight gain. Also there were the significant effects at a higher dose.

Then we measured blood pressure and heart pulse rate (Fig. 3). Treatment with adenine significantly increased blood pressure (143±4.2 mmHg) and inhibited heart rates (403±2.6 beats/min) (*p*<0.001 and *p*<0.01) in comparison to normal group (127±5.7 mmHg and 436±1.7 beats/min). SGW-100 and SGB-50 significantly reduced blood pressure to 133±2.9 and 131±2.2 mmHg (*p*<0.01), while they significantly inhibited heart rates decreased by adenine (432±1.4 and 433±2.0 beats/min) (*p*<0.001).

In the present study, we investigated the protective effect of SG total saponin fraction against adenine-induced chronic renal failure in rats. It has been reported that the intake of adenine induced extraordinary increases of creatinine and urea nitrogen in the serum by decreasing the excretion of these substances. Dietary adenine also caused a reduction of serum calcium level and an increase of serum phosphate level. As a result, SGB as well as SGW significantly decreased the levels of both urea nitrogen and creatinine in the serum. And, it significantly

increased the urinary excretions of both urea nitrogen and creatinine. Furthermore, the administration of SGW or SGB increased serum calcium levels indicating an improvement of hypocalcemia, and decreased serum phosphate levels indicating an improvement of hyperphosphatemia. Treatment with SGW or SGB inhibited adenine-induced kidney hypertrophy or edema as well as liver injury. SGB more potently ameliorated adenine-induced renal failure than SGW. Based on these findings, SG may improve CRD and its effect may be dependent on its main ingredient, ginseng saponin.

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