Germanium in ginseng is low and causes no sodium and water retention or renal toxicity in the diuretic-resistant rats

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Abstract

Ginseng preparations contain high concentrations of germanium (Ge), which was reported to contribute to diuretic resistance or renal failure. However, Ge content in ginseng and the influence on renal functions remain unclear. Forty rats were randomly divided into control group, low, moderate, and high Ge ginseng-treated group and observed for 25 days. Daily urine, renal functions, and serum and urine electrolytics were measured. Ge retention in the organs and renal histological changes were also evaluated. Ge content ranged from 0.007 to 0.450 $\mu\text{g/g}$ in various ginseng samples. Four groups showed no difference in the daily urine output, glomerular filtration rate, urinary electrolytes excretions, 24 h-urine protein, as well as plasma and urine urea nitrogen, creatinine, osmotic pressure, and pH values. Ge did not cause any renal pathological effects in this study. No Na and water retention was detected in the ginseng-treated groups. Ge retention in various organs was found highest in spleen, followed by the kidney, liver, lung, stomach, heart, and pancreas. The total Ge contents in various ginsengs were low, and ginseng treatment did not affect renal functions or cause renal histological changes.

Keywords: Germanium in ginseng, sodium and water retention, renal toxicity, diuretic-resistant rats

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Introduction

Ginseng has been used widely as a tonic in China for several thousands of years, however, recent studies report that some ginseng preparations contain high concentrations of germanium (Ge), which contributes to diuretic resistance or renal failure.^{1,2} Thus, the contents of Ge in ginseng as well as the influence of ginseng on renal functions are of great concern.

Most of the previous cases, however, were associated with other diseases and/or the intake of additional drugs, making it difficult to determine whether the effects were caused by the Ge in ginseng only. Thus, these requirements prompted us to investigate the Ge contents in various ginseng samples that were obtained from different areas and growth periods. We treated the animals with the various ginsengs containing different concentrations of Ge, and detected the levels of Ge in plasma and urine, Ge retention in various organs, and also evaluated the renal functions and histological changes. Therefore, the current experiment is to determine the accurate levels of ginseng Ge from various countries, and to investigate the potential adverse effects of ginseng Ge on the renal functions and the pathological changes in the kidney of the rats.

Materials and methods

Reagents and apparatus

Unless otherwise stated, all reagents used in the experiments were analytical-grade chemicals dissolved in deionized water. A 1000 mg L^{-1} stock solution of inductively coupled plasma – grade Ge was purchased from Reagacon (Shannon, Ireland) and used as the standard sample. Working solutions were prepared by appropriate dilution of this standard stock solution. Concentrated sulfuric, nitric, and hydrochloric acids (Sigma Aldrich, Ireland) were used for the digestion of ginseng samples.

Absorbance was monitored using a Varian GTA 110 instrument equipped with a programmable sample dispenser (PSD 55) and a coated graphite partition tube (Varian) with wall atomization. The radiation source was a Ge hollow cathode lamp. Furnace analysis conditions for graphite furnace atomic absorption spectroscopy (GFAAS) are shown in Table 1.

Determination of Ge contents in different ginseng samples

Ginseng samples grown from 3 to 15 years in different regions were obtained from the local drug stores and five

kinds of ginseng samples in the same origin and same growing periods were detected. The samples were dehydrated and ground to a fine powder, after which the Ge content in each sample was detected by GFAAS, as previously described.³ Briefly, 0.1 g of ginseng powder was transferred to an evaporating dish and mixed with 10 mL of nitric acid. The digest was boiled and the volume was reduced to 1–2 mL. Perchloric acid (1 mL) was added to this solution, and the digest was reduced to dryness. A 20-mL volume of deionized water was added, and the mixture was again reduced to almost dryness and transferred to a 50 mL volumetric flask. The Ge content in each sample was subsequently detected by GFAAS. The limit of quantification was determined to be 20 µg/L or 0.3 ng of Ge based on a 15 µL injection.

Rats treated with various ginseng samples

Sprague–Dawley male rats weighing 300–400 g were housed in metabolic cages (Tecniplast, Varese, Italy) under a controlled temperature ($22 \pm 1^\circ\text{C}$). Rats were acclimatized for seven days before the experiments and allowed water and food *ad libitum*. All of the animals were injected with furosemide (1.5 mg twice a day intraperitoneally for four days) and sacrificed as previously described.⁴ Significant diuresis and natriuresis were observed on the first two days of induction. By the fourth day, renal excretion returned to basal levels despite repeated furosemide

treatment. Thus, the diuretic-resistant rat model was considered to be successfully established for the experiments.

The rats were then divided into four groups in a randomized manner ($n=10$ in each group). Three of the groups were given ginseng powder at 2 g/kg/day in 1 mL of deionized water by daily oral gavage. The ginseng solutions contained high (0.450 ± 0.025 µg/g), moderate (0.250 ± 0.023 µg/g), or low Ge (0.007 ± 0.001 µg/g) contents. The control group was given an equivalent concentration of wheat flour in deionized water. All animals were caged separately for 25 days. Blood samples were collected by jugular vein puncture under light ether anesthesia every five days at approximately 10 a.m., and urine was collected daily. The study was approved by the local animal care committee of the university.

Measurements of glomerular filtration rate (GFR)

GFR was measured as inulin clearance during constant intraperitoneal infusion of radiolabeled inulin with osmotic minipumps, as described previously.⁵ Minipumps (Model 2001; Alza Corp., Palo Alto, CA) were filled with an aqueous solution containing 250 µCi/mL of [carboxyl-¹⁴C] inulin (ICN, Costa Mesa, CA). These were implanted into the peritoneal cavity under ether anesthesia and delivered at 24 µL/day with approximately 10×10^6 cpm/day of ¹⁴C-inulin. Radioactivity in plasma and urine samples was measured by liquid scintillation with a Picofluor 30 (LKB Compugamma, Wallac Oy, Finland). The radioactivity in the plasma samples (30 µL) was about 10 times that of the background.

Ge concentrations in serum and urine and its retention in organs

About 100 mg of lung, heart, spleen, stomach, pancreas, liver, and kidney tissues, 400 µL of plasma, and 1 mL of urine were accurately weighed in demetalolyzed Pyrex

Table 1 Graphite furnace analytical conditions (time in seconds)

Step/time	85°C	95°C	120°C	700°C	2600°C	2800°C
Drying	35 s	45 s	15 s			
Ashing				10 s		
Atomization					4 s	
Tube clean						3 s

Table 2 Total Ge content in various ginsengs detected by GFAAS (µg/g)

Areas	Growing periods			
	3–5 years	6–10 years	11–15 years	>15 years
Japan ($n=5$)	0.007 ± 0.001	0.018 ± 0.005	0.021 ± 0.009	$0.037 \pm 0.011^*$
North Korea ($n=5$)	0.011 ± 0.003	0.024 ± 0.007	0.058 ± 0.004	$0.250 \pm 0.023^*$
South Korea ($n=5$)	0.015 ± 0.004	0.028 ± 0.006	0.176 ± 0.011	$0.318 \pm 0.032^*$
China				
Heilongjiang ($n=5$)	$0.110 \pm 0.020\#$	$0.220 \pm 0.009\#$	$0.320 \pm 0.021\#$	$0.450 \pm 0.025\#^*$
Liaoning ($n=5$)	0.012 ± 0.006	0.021 ± 0.004	0.031 ± 0.006	$0.350 \pm 0.016^*$
Jilin ($n=5$)	0.093 ± 0.007	0.130 ± 0.007	0.230 ± 0.021	$0.380 \pm 0.037^*$
Hebei ($n=5$)	0.047 ± 0.006	0.161 ± 0.011	0.287 ± 0.011	$0.340 \pm 0.042^*$
Canada ($n=5$)	0.023 ± 0.008	0.047 ± 0.006	0.075 ± 0.006	$0.187 \pm 0.011^*$
America ($n=5$)	0.020 ± 0.005	0.043 ± 0.005	0.067 ± 0.007	$0.277 \pm 0.021^*$
Russia ($n=5$)	0.033 ± 0.004	0.057 ± 0.006	0.145 ± 0.011	$0.365 \pm 0.031^*$

Note: GFAAS, graphite furnace atomic absorption spectroscopy. The total Ge content in various ginsengs varied from region to region, and the ginseng grown in the same region was linearly correlated with growth length.

# $P < 0.05$ compared to the ginsengs from different regions in the same growth period. * $P < 0.05$ compared to the ginsengs in 6–11 growth years or in 11–15 growth years.

Table 3 Renal functions responses to the different ginseng samples treatment

Variables groups	Day	DUO (mL/24 h)	GFR (mL/min)	Na (mmol/mL)	Cl (mmol/mL)	K (mmol/mL)	UUN (mmol/mL)	Ucr (mmol/mL)	Uosm (mosm/kg H ₂ O)	UP (24h)	pH	Weight (g)
Control (n = 10)	0	6.34 ± 2.21	2.33 ± 0.21	1.12 ± 0.11	1.62 ± 0.12	0.89 ± 0.15	0.88 ± 0.21	2.78 ± 0.42	761 ± 41	0.62 ± 0.11	7.13 ± 0.02	345 ± 45
	5	6.56 ± 2.32	2.12 ± 0.14	1.22 ± 0.12	1.66 ± 0.14	0.85 ± 0.11	0.82 ± 0.22	2.66 ± 0.32	752 ± 31	0.63 ± 0.12	7.22 ± 0.05	348 ± 42
	10	7.12 ± 1.34	2.62 ± 0.17	1.15 ± 0.14	1.73 ± 0.14	0.91 ± 0.20	0.87 ± 0.18	2.57 ± 0.22	768 ± 45	0.71 ± 0.14	7.31 ± 0.07	351 ± 37
	15	6.66 ± 2.21	2.45 ± 0.22	1.23 ± 0.10	1.68 ± 0.23	0.82 ± 0.12	0.92 ± 0.23	2.85 ± 0.32	782 ± 31	0.69 ± 0.17	7.15 ± 0.05	355 ± 25
	20	7.22 ± 1.21	2.52 ± 0.18	1.42 ± 0.15	1.57 ± 0.17	0.92 ± 0.17	0.79 ± 0.11	2.66 ± 0.32	772 ± 44	0.73 ± 0.18	7.21 ± 0.12	361 ± 35*
Low Ge (n = 10)	25	6.78 ± 1.81	2.63 ± 0.24	1.33 ± 0.13	1.75 ± 0.18	0.88 ± 0.13	0.93 ± 0.25	2.69 ± 0.24	778 ± 33	0.71 ± 0.15	7.18 ± 0.11	372 ± 47*
	0	6.45 ± 2.42	2.52 ± 0.21	1.23 ± 0.21	1.59 ± 0.13	0.87 ± 0.18	0.82 ± 0.11	2.56 ± 0.34	772 ± 32	0.67 ± 0.13	7.22 ± 0.03	351 ± 25
	5	6.37 ± 2.31	2.45 ± 0.23	1.28 ± 0.22	1.61 ± 0.15	0.83 ± 0.14	0.85 ± 0.13	2.52 ± 0.33	775 ± 36	0.71 ± 0.15	7.18 ± 0.04	357 ± 23
	10	6.51 ± 2.55	2.57 ± 0.22	1.32 ± 0.19	1.66 ± 0.12	0.91 ± 0.17	0.87 ± 0.12	2.66 ± 0.36	768 ± 30	0.68 ± 0.12	7.21 ± 0.02	360 ± 21
	15	6.71 ± 2.81	2.71 ± 0.19	1.33 ± 0.23	1.70 ± 0.15	0.88 ± 0.15	0.84 ± 0.13	2.58 ± 0.37	768 ± 28	0.77 ± 0.15	7.22 ± 0.04	365 ± 23
Moderate Ge (n = 10)	20	6.77 ± 2.63	2.63 ± 0.11	1.37 ± 0.25	1.69 ± 0.11	0.85 ± 0.12	0.82 ± 0.13	2.71 ± 0.38	776 ± 37	0.69 ± 0.12	7.15 ± 0.04	370 ± 28*
	25	6.67 ± 2.52	2.68 ± 0.23	1.41 ± 0.17	1.65 ± 0.14	0.92 ± 0.19	0.86 ± 0.15	2.76 ± 0.38	781 ± 33	0.72 ± 0.14	7.18 ± 0.03	373 ± 29*
	0	6.28 ± 2.35	2.33 ± 0.21	1.17 ± 0.11	1.65 ± 0.11	0.81 ± 0.12	0.85 ± 0.22	2.68 ± 0.44	759 ± 45	0.58 ± 0.13	7.18 ± 0.04	339 ± 22
	5	6.32 ± 2.45	2.43 ± 0.21	1.22 ± 0.13	1.71 ± 0.13	0.91 ± 0.16	0.92 ± 0.25	2.72 ± 0.32	762 ± 31	0.61 ± 0.12	7.24 ± 0.02	345 ± 35
	10	6.36 ± 2.27	2.37 ± 0.21	1.19 ± 0.15	1.68 ± 0.12	0.87 ± 0.12	0.89 ± 0.31	2.69 ± 0.41	767 ± 21	0.65 ± 0.17	7.23 ± 0.04	351 ± 22
High Ge (n = 10)	15	6.42 ± 2.33	2.57 ± 0.21	1.23 ± 0.12	1.72 ± 0.14	0.93 ± 0.15	0.85 ± 0.25	2.75 ± 0.41	771 ± 33	0.60 ± 0.14	7.21 ± 0.03	365 ± 15
	20	6.37 ± 2.44	2.53 ± 0.21	1.32 ± 0.14	1.73 ± 0.15	0.89 ± 0.13	0.87 ± 0.23	2.77 ± 0.32	772 ± 28	0.57 ± 0.16	7.21 ± 0.07	370 ± 25*
	25	6.34 ± 2.31	2.62 ± 0.21	1.25 ± 0.16	1.69 ± 0.17	0.86 ± 0.12	0.92 ± 0.23	2.68 ± 0.32	768 ± 36	0.64 ± 0.12	7.24 ± 0.05	375 ± 35*
	0	6.52 ± 2.32	2.25 ± 0.17	1.24 ± 0.12	1.65 ± 0.14	0.78 ± 0.13	0.85 ± 0.19	2.56 ± 0.32	756 ± 31	0.58 ± 0.12	7.23 ± 0.01	338 ± 25
	5	6.48 ± 2.45	2.42 ± 0.25	1.31 ± 0.13	1.71 ± 0.12	0.85 ± 0.12	0.76 ± 0.23	2.65 ± 0.21	762 ± 22	0.61 ± 0.13	7.30 ± 0.05	345 ± 22
	10	6.63 ± 2.28	2.37 ± 0.22	1.28 ± 0.12	1.72 ± 0.15	0.83 ± 0.15	0.77 ± 0.21	2.48 ± 0.33	758 ± 24	0.59 ± 0.11	7.16 ± 0.04	350 ± 31
	15	6.78 ± 2.37	2.41 ± 0.24	1.25 ± 0.14	1.68 ± 0.11	0.91 ± 0.17	0.82 ± 0.25	2.75 ± 0.24	764 ± 33	0.63 ± 0.12	7.23 ± 0.02	355 ± 28
	20	6.86 ± 2.67	2.51 ± 0.27	1.22 ± 0.11	1.64 ± 0.12	0.79 ± 0.16	0.87 ± 0.24	2.65 ± 0.22	771 ± 27	0.71 ± 0.15	7.23 ± 0.01	363 ± 27*
	25	6.78 ± 2.56	2.48 ± 0.32	1.24 ± 0.13	1.77 ± 0.15	0.75 ± 0.12	0.91 ± 0.11	2.78 ± 0.32	764 ± 35	0.66 ± 0.13	7.25 ± 0.02	372 ± 35*

Note: DUO, daily urine output; GFR, glomerular filtration rate; electrolytes excretions (Na, K, Cl), UUN, urine urea nitrogen; Ucr, urine creatinine; UP/24h, 24 h-urine protein; Uosm, urine osmotic pressure and urine pH values exhibited no statistical difference within each group or among the four groups ($P > 0.05$). Body weight in all groups showed significantly higher values on days 25 and 30 compared to day 0 ($P < 0.05$ vs. the baseline 0 day in each group).

Table 4 Plasma values at baseline and every five days

Variables groups	Day	Na (mmol/mL)	Cl (mmol/mL)	K (mmol/mL)	BUN (mmol/mL)	Scr (μmol/mL)	P _{osm} (mosm/kg H ₂ O)	pH	Serum Ge	Urine Ge
Control (n = 10)	Baseline	135 ± 8	103 ± 5	4.3 ± 0.2	4.21 ± 0.21	41.2 ± 1.2	312 ± 2	7.13 ± 0.02	< 0.001	< 0.001
	5	133 ± 5	108 ± 6	4.2 ± 0.1	4.17 ± 0.32	42.1 ± 1.3	314 ± 6	7.21 ± 0.02	< 0.001	< 0.001
	10	131 ± 4	107 ± 4	3.8 ± 0.4	4.12 ± 0.42	39.52 ± 1.7	321 ± 3	7.23 ± 0.03	< 0.001	< 0.001
	15	137 ± 6	110 ± 7	3.7 ± 0.3	4.45 ± 0.17	42.7 ± 2.1	318 ± 2	7.28 ± 0.04	< 0.001	< 0.001
	20	141 ± 9	105 ± 5	4.1 ± 0.2	3.87 ± 0.28	38.8 ± 1.7	320 ± 5	7.24 ± 0.12	< 0.001	< 0.001
Low Ge (n = 10)	25	138 ± 7	111 ± 8	3.9 ± 0.1	4.35 ± 0.36	43.1 ± 2.2	316 ± 7	7.27 ± 0.11	< 0.001	< 0.001
	Baseline	129 ± 6	111 ± 6	3.8 ± 0.3	4.34 ± 0.35	42.18 ± 1.7	321 ± 4	7.25 ± 0.03	< 0.001	< 0.001
	5	132 ± 6	109 ± 8	3.5 ± 0.4	4.12 ± 0.25	39.7 ± 1.4	318 ± 7	7.32 ± 0.02	0.002 ± 0.001	0.001 ± 0.001
	10	135 ± 5	113 ± 7	4.1 ± 0.5	3.78 ± 0.33	37.8 ± 2.2	325 ± 9	7.25 ± 0.03	0.003 ± 0.001	0.003 ± 0.001
	15	131 ± 7	111 ± 5	3.8 ± 0.1	3.68 ± 0.24	42.1 ± 3.1	318 ± 6	7.24 ± 0.04	0.004 ± 0.001	0.005 ± 0.001
Moderate Ge (n = 10)	20	133 ± 2	107 ± 6	4.2 ± 0.2	4.41 ± 0.34	43.1 ± 4.2	323 ± 9	7.23 ± 0.03	0.006 ± 0.002	0.007 ± 0.002*
	25	131 ± 8	112 ± 8	4.1 ± 0.3	4.22 ± 0.21	38.7 ± 3.3	315 ± 7	7.13 ± 0.03	0.007 ± 0.004	0.009 ± 0.002*
	Baseline	131 ± 7	101 ± 7	3.8 ± 0.7	3.67 ± 0.21	39.6 ± 2.5	322 ± 6	7.26 ± 0.02	< 0.001	< 0.001
	5	137 ± 10	112 ± 8	3.5 ± 1.1	4.07 ± 0.21	37.8 ± 2.5	319 ± 7	7.18 ± 0.02	0.004 ± 0.001	0.004 ± 0.001
	10	129 ± 11	109 ± 5	3.8 ± 0.8	3.81 ± 0.21	40.6 ± 2.5	323 ± 9	7.29 ± 0.03	0.007 ± 0.001	0.007 ± 0.001
High Ge (n = 10)	15	131 ± 8	113 ± 7	4.1 ± 1.2	3.76 ± 0.21	37.5 ± 2.5	325 ± 5	7.31 ± 0.03	0.009 ± 0.002	0.011 ± 0.002
	20	129 ± 7	107 ± 9	3.9 ± 0.5	4.12 ± 0.21	38.7 ± 2.5	318 ± 5	7.28 ± 0.05	0.010 ± 0.005	0.014 ± 0.005*
	25	132 ± 12	111 ± 6	4.1 ± 1.2	4.11 ± 0.21	41.2 ± 2.5	320 ± 7	7.32 ± 0.02	0.009 ± 0.007	0.021 ± 0.004*
	Baseline	131 ± 8	108 ± 7	3.8 ± 0.7	3.76 ± 0.21	38.7 ± 1.2	321 ± 5	7.25 ± 0.01	< 0.001	< 0.001
	5	128 ± 7	110 ± 8	4.1 ± 1.2	3.55 ± 0.21	40.5 ± 1.5	316 ± 7	7.32 ± 0.02	0.005 ± 0.001	0.008 ± 0.001
	10	133 ± 9	109 ± 5	3.6 ± 0.7	4.11 ± 0.21	41.4 ± 1.7	322 ± 8	7.25 ± 0.04	0.008 ± 0.002	0.012 ± 0.007
	15	129 ± 5	111 ± 9	4.0 ± 0.7	3.87 ± 0.21	38.5 ± 1.1	323 ± 6	7.33 ± 0.03	0.010 ± 0.002	0.018 ± 0.006
	20	133 ± 6	112 ± 7	3.8 ± 1.1	4.12 ± 0.21	37.7 ± 1.4	318 ± 4	7.23 ± 0.01	0.011 ± 0.007	0.027 ± 0.009*
	25	131 ± 7	100 ± 5	4.1 ± 0.8	4.11 ± 0.21	42.1 ± 1.2	324 ± 7	7.15 ± 0.03	0.012 ± 0.007	0.038 ± 0.012*

Note: BUN, serum Na, K, Cl, blood urea nitrogen; Scr, serum creatinine; Posm, plasma osmotic pressure and blood pH values, as well as serum Ge, exhibited no statistical difference within each group or among the four groups at all time points studied ($P > 0.05$). Ge urinary excretions were significantly higher in ginseng group at day 20 and 25 than the baseline or at day 5, and Ge excretions showed a linear correlation with the animals intake. (* $P < 0.05$ vs. baseline or day 5 in ginseng group).

Table 5 Ge's organ retention in the different groups ($\mu\text{g/g}$)

Groups	Lung	Heart	Liver	Stomach	Pancreas	Spleen	Kidney
Control ($n = 10$)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Low Ge ($n = 10$)	0.001 ± 0.001	0.001 ± 0.001	0.004 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.008 ± 0.002	0.006 ± 0.001
Moderate Ge ($n = 10$)	0.025 ± 0.003	0.012 ± 0.002	0.120 ± 0.003	0.018 ± 0.002	0.011 ± 0.002	0.212 ± 0.001	0.170 ± 0.007
High Ge ($n = 10$)	0.046 ± 0.002	0.018 ± 0.002	0.172 ± 0.004	0.024 ± 0.002	0.012 ± 0.002	$0.342 \pm 0.011\#^*$	$0.283 \pm 0.005^*$

Note: The retention of Ge in the organs was correlated with the animals' intake. The highest Ge retention was found in spleen, followed in descending order kidney, liver, lung, stomach, heart, and pancreas in the three ginseng-treated groups.

# $P < 0.05$ compared to any other organ in high Ge group; * $P < 0.05$ versus the moderate Ge group.

glass tubes. The amounts of Ge in these samples were detected by flow injection GFAAS with minor modifications.⁶ The samples were heated to 120–140°C with 2 mL of HNO_3 and 0.2 mL of HClO_4 in a block bath (Advantec, Tokyo, Japan) to digest organic components and subsequently diluted with 0.5% HNO_3 . Ge contents in each sample were calculated using a matrix-matched calibration curve containing corresponding amounts of the specimen obtained from the controls, as described previously.⁶ Working solutions were prepared from the Ge standard solution by dilution with 0.5% HNO_3 . The whole system was controlled by a dedicated computer software program that allowed fully automated analytical runs of the samples and internal standards.

Other measurements

Rats were weighed every five days, and daily urine output was collected in flasks containing mineral oil and thymol. Standard techniques were used to measure volume, Na, K, and Cl concentrations, as well as blood and urine pH. Urine and plasma osmolality were measured by a freezing point osmometer (Roebeling, Berlin, Germany). Concentrations of creatinine and urea nitrogen in the urine and plasma were measured every five days by a Hitachi 917 analyzer (Roche Diagnostics).

Histopathological studies

Renal weight and histopathology were evaluated. Tissues were fixed in 4% phosphate-buffered formalin solution. Cross sections of 5–6 μm were cut and slices were routinely stained with hematoxylin and eosin. The renal medulla and cortex were assessed under a light microscope (Nikon Eclipse E400). The following criteria were used for scoring kidney histology: + + + +, a change was very often found in the tissue; + + +, relative common in the tissue; + +, a few in the tissue; +, sporadic in the tissue; and – or $\pm$, rare in the tissue.

Data analysis

Data are reported as $M \pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison tests were used to evaluate differences between groups. To evaluate differences among different

periods within the same group, one-way ANOVA followed by Newman–Keuls tests were used. $P < 0.05$ was considered to be statistically significant.

Results

Ge contents are low in various ginseng samples

GFAAS showed that the concentrations of Ge in the ginseng samples grown in Japan, Korea, America, Canada, and Russia ranged from $0.007 \pm 0.001 \mu\text{g/g}$ in three to five growth years to $0.450 \pm 0.025 \mu\text{g/g}$ in over 15 growth years. Japanese ginseng extracts showed the lowest (0.007 ± 0.001 – $0.037 \pm 0.011 \mu\text{g/g}$) among samples obtained from the five countries. By contrast, Chinese ginseng samples generally contained higher levels of Ge than those from other countries; the highest Ge was found in Heilongjiang-cultivated ginseng. The Ge contents in various ginseng samples were linearly correlated with the growth and varied significantly from region to region (Table 2). Previous data showed the total Ge in ginseng root in the range of 0.35–0.41 $\mu\text{g/g}$,⁷ the Ge contents were comparable with tomato, bamboo shoot, potato, and other vegetables. Ours and previous data confirmed that the ginseng samples generally contain low levels of Ge. Thus, the high Ge content observed in commercial ginseng preparations (as mentioned in Sanai et al.¹) cannot stem from the ginseng plant itself, but an artificial supplementation.

Renal functions are unaffected by various ginseng treatments

To observe the response to ginseng Ge, renal functions were detected at the baseline (0 day) and every five days thereafter for a period of 25 days in each group. The parameters at baseline were comparable among the four groups (Table 3). Daily urine output, GFR, renal excretions of Na, Cl, and K, as well as urine urea nitrogen, urine creatinine, 24-h urine protein, and urine osmotic pressure and urine pH values, showed no difference within each group or among the four groups. The body weight in ginseng groups was similar to the control group. These data suggest that ginseng with various Ge did not influence renal functions or cause Na and water retention in rats.

Figure 1 Epithelial cells and renal tubules were evident in ginseng-treated or control rats (a,c,e,g). High magnification showed mild dilation of glomeruli with few mononuclear cell infiltrates (b,d,f,h). Paraffin sections of the tissue were stained with H&E. Scale = 100 μ m. (a,b) control group; (c,d): rats treated with low Ge ginseng; (e,f): moderate Ge ginseng; (g,h): high Ge ginseng (a,c,e,g) 200 $\times$; (b,d,f,h, 400 $\times$). (A color version of this figure is available in the online journal).

Ge is excreted in the urine and ginseng treatment did not affect serum electrolytes

Serum Na, Cl, and K as well as serum urea nitrogen, serum creatinine, plasma osmotic pressure, and pH values, showed no statistical difference within each group or

among the four groups. Furthermore, serum Ge remained stable and low despite treatment with the high Ge ginseng samples. Ge urinary excretion was corresponding with ginseng Ge intake, suggesting the renal handling of Ge rapidly and excreted mainly through kidney. The data were found in line with previous data⁸ (Table 4).

Table 6 Histopathological changes in kidney of rats treated with various ginsengs

Finding	Control	Treated groups		
		Low	Moderate	High
Hypertrophy of epithelial cells of renal tubules	±	±	+	+
Degeneration of tubular epithelial with simultaneous infiltration of mononuclear cells	+	+	+	+
Hyperaemia of medullary and cortical part with mononuclear cell infiltration	+	+	+	+
Dilatation of renal glomeruli	±	+	±	+

Note: Animals were exposed to various ginsengs treatment for 25 days. Pathological changes were scored on a ± (rare in the tissue) to + (sporadic in the tissue). The study was done on 10 rats in each group.

Ge retention is low and causes no renal parenchyma injury

To determine Ge retention, Ge contents in the spleen, kidney, liver, lung, stomach, heart, and pancreas were calculated using a matrix-matched calibration curve that contained corresponding amounts of control specimens. The highest Ge contents were found in the spleen, followed by the kidney, liver, lung, stomach, heart, and pancreas. By contrast, Ge concentrations were very low and hardly detectable in control as shown in Table 5.

The renal cortex and medulla, as well as the tubules and glomeruli in the control rats (Figure 1(a)) exhibited the similar structure in the various ginseng-treated rats (Figure 1(c,e,g)). Examination at high magnification showed that mild dilation of glomeruli and hyperemia of medullary and cortical parts with few mononuclear cells infiltrates in the control and ginseng-treated rats (Figure 1(b,d,f,h)), however, the three ginseng-treated groups and the control group showed the similar pathological grades in cortex and medulla as shown in Table 6.

Discussion

The amounts of Ge in ginseng depend on soil, water, and air conditions in the growth area, however, Ge mainly originates from soil and water. In most land areas in the world, Ge contents in soil and natural water are approximately 0.6–1.3 mg/kg (atmosphere 3 µg/m³), and ginseng's enrichment ratio of Ge from the soil and water is approximately 50%.⁹ Therefore, the maximum theoretical amount of Ge in ginseng should be no more than 1 mg/kg, which is confirmed in the current experiments. Furthermore, when the Ge content in the soil reaches 5 mg/kg, the soil becomes highly toxic to most plants, including ginseng.¹⁰ Despite inherent differences among growth areas and periods, the Ge content in the ginseng samples obtained was generally too low to cause renal dysfunction in the diuretic-resistant rat models. Rats treated with different ginseng preparations showed results similar to those of control rats in terms of daily urine output, electrolytes excretions, GFR, urine urea nitrogen, urine creatinine, 24-h urine protein, urine osmotic pressure, and urine pH values.

Plasma electrolytes, serum urea nitrogen, serum creatinine, plasma osmotic pressure, and pH values did not statistically differ between the various ginseng-treated animals and control animals. Furthermore, the concentrations of

serum Ge were low and stable despite treatment with the Ge ginseng preparations. Ge was excreted mainly through the urine and its retention in the organs was very low. As such, no adverse effects on the renal functions and its histology were observed in the current experiments. The body weight of the various ginseng-treated animals was comparable with the control animals, which suggests that ginseng treatment does not exacerbate Na and water retention. This finding is inconsistent with a previous report that ginseng could induce Na and water retention in a renal failure patient.² As the renal failure patient received more than one drug during treatment, drug-ginseng interactions may exacerbate the conditions of renal toxicity. In addition, urea and many other metabolic end products retained in the blood may interact with ginseng, causing various adverse effects, including diuretic resistance. Our experiments showed no association between ginseng Ge and renal toxicity.

Although ginseng has been used as a tonic in China for thousands of years, it is not considered a cure for all diseases or suitable in all conditions. For example, ginseng has many contraindications, including hypertension, particularly when the systolic blood pressure ≥180 mmHg, with the symptoms of edema due to damp and heat and renal insufficiency with anuria or oliguria as differentiated by the Traditional Chinese Medicine (TCM). Thus, the use of ginseng should follow theories of individualization and differentiation in TCM. Empirically, TCM recommends 5–10 g/day of ginseng for common dosage and 15–30 g/day of the plant for stem vacuity desertion. The ginseng dosage used in current experiments was equivalent to 15–30 g/day for a 60-kg adult person.¹¹ Such a high dosage of ginseng, however, did not cause Na and water retention, nor renal toxicity was observed in current experiments. Nevertheless, administration of the proper indications and dosages of ginseng and its compounds for humans under the guidance of a doctor is advised.

As an ultra-trace element, Ge contents in food are very low, and concentrations of the mineral are generally suitable for humans.^{12,13} To date, no data support the belief that Ge is an essential trace element for humans. Food supplements containing Ge have been voluntarily withdrawn in the United Kingdom and are banned in the United States because of its association with several diseases, such as renal failure, gastrointestinal disturbances, liver dysfunction, anemia, neuropathy, and even death.^{14,15,1,16} However, some reports claimed that Ge supplements can

prevent or treat cancer, AIDS, senile osteoporosis, liver disease, hypertension, arthritis, malaria, food allergies, hypercholesterolemia, and myalgic encephalomyelitis.⁸ In some countries, Ge is still considered a nutritional supplement because of its purported immunomodulatory effects and health benefits. These claims have prompted some manufacturers to add large amounts of Ge to ginseng preparations as an additive, which causes Ge consumption to exceed average dietary requirements, and ginseng has become a scapegoat. Nevertheless, more experiments are necessary to determine whether the mineral has therapeutic effects. Our data show that various ginsengs contain very low Ge concentrations, and that normal dosages of ginseng do not cause renal dysfunction at least on a short-term basis. Future studies are required to determine the long-term (months to years) effects of ginseng consumption.

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